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Cultural evolution in Rufous-and-white Wrens (Thryophilus rufalbus): Insights from acoustic and genetic analyses

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CULTURAL EVOLUTION IN RUFOUS-AND-WHITE WRENS (*THRYOPHILUS RUFALBUS*): INSIGHTS FROM ACOUSTIC AND GENETIC ANALYSES

by

Brendan Alan Graham

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Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows: I am the sole author of the General Introduction (Chapter 1) and the General Discussion (Chapter 7). I am the principle author of Chapters 2 through Chapter 6. All five of these chapters are co-authored by my supervisor, Dr. Daniel Mennill. Chapters 3 through 6 are co-authored by our collaborator Dr. Daniel Heath, Chapters 3 through 5 are co-authored by Dr. Ryan Walter, Chapter 2 is co-authored by Dr. Torben Dabelsteen and Dr. Luis Sandoval, and Chapter 3 is co-authored by Dr. Melissa Mark. In all five instances the key ideas are mine, and the experimental design, experimental execution, data analysis, interpretation, and writing were performed primarily by me. Dr. Daniel Mennill contributed to experimental design, data analysis, interpretation, and the writing and editing of the manuscripts, as well as providing financial and logistical assistance for the research described in all five data chapters. My other coauthors contributed to the experimental design, data collection, data interpretation, and/or editing and writing of the manuscripts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

Chapter 2 is published in the journal Bioacoustics. Chapters 3, 4, 5, and 6 are written in manuscript format for future submission to scientific journals.

II. Declaration of Previous Publication

This thesis includes one original paper that has been previously published in a peer-reviewed journal, as follows:

Thesis Chapter	Publication title/full citation	Publication status*
Chapter 2	A test of the Acoustic Adaptation Hypothesis in three types of tropical forest: degradation of male and female Rufous-and-white Wren songs	Published in *Bioacoustics*

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Abstract

Acoustic signals play an important role in the evolutionary process. Studying variation in acoustic signals and the evolutionary forces that act on acoustic evolution will help to clarify the role that acoustic divergence plays during speciation. The acoustic signals of birds have been well studied, although most historical research has focused on the acoustic traits of males living at temperate latitudes. Recent work shows that female song is more common than previously thought, particularly in the tropics, and that female song is the ancestral trait in birds. Therefore further research on the acoustic traits of females is necessary to examine the evolutionary significance of animal vocal signals. In this dissertation I compare patterns of cultural evolution in male and female Rufous-and-white Wrens, and I examine the evolutionary forces that act on male and female acoustic signals. To achieve this goal, I combine acoustic and genetic analysis to quantify genetic differentiation, migration, and dispersal patterns and determine the role that these factors have on acoustic variation. In addition I incorporate ecological data and use a sound transmission experiment to explore the effect that ecological variation has on acoustic evolution. My results indicate that males and females exhibit similar patterns of acoustic variation, suggesting that similar evolutionary processes act on both male and female songs. Specifically, acoustic differences between populations appear to arise due to cultural drift or cultural selection, as opposed to genetic variation and ecological selection, as has been shown in other species. Males and females also show cultural differences, including lower song-sharing rates by females, greater inter-annual variation in the acoustic structure of female songs, and sound transmission differences. These patterns indicate that there may be sex-based differences in selection pressures acting on songs. Additionally, my results show that dispersal is female-biased in Rufous-and-white Wrens and therefore cultural differences between sexes may arise as a result of dispersal differences between the sexes.

Collectively, my results provide further insight into the acoustic variation of male and female birds, and expand our knowledge of female song and the vocalizations of tropical animals.

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Chapter 1: General Introduction

Introduction

Animals use a wide diversity of signals to attract mates and defend resources, including acoustic, chemical, electrical, vibrational, and visual signals (Bradbury and Vehrencamp, 2011). Signals often vary spatially, and therefore a central area of evolutionary biology has focused on the forces that underlie geographic variation, signal divergence, and their role in speciation (Endler, 1992; Coyne and Orr, 2004). Do traits diverge between populations and species due to ecological and genetic differences, or through the influence of sexual selection (McDonald *et al*., 2001; Boughman, 2002; Ruegg *et al*., 2006; Wang *et al*., 2010; Seddon *et al*., 2013)? Integrative studies that incorporate multiple analyses to examine how signals vary and evolve allow us to further evaluate the processes that contribute to the evolution of animal signals (Slabbekoorn and Smith, 2002; Wilkins *et* al., 2013; Kemp *et al*., 2015).

Particular interest has focused on variation in acoustic signals (Bradbury and Vehrencamp, 2011). These signals often vary among geographic locations (Mitani *et al*., 1992; Cerchio *et al*., 2001; Prohle *et al*., 2006; Podos and Warren, 2007; Campbell *et al*., 2011), and given the important role that they play in resource defense of mate attraction, acoustic signals may act as reproductive isolating barriers and play a role in speciation (Irwin *et al*., 2001; Patten *et al*., 2004; Edwards *et al*., 2005; Wilkins *et al*., 2013). Many factors are thought to influence the evolution of acoustic signals, including environmental, morphological, phylogenetic, and physiological constraints, as well as selective forces resulting from differences in predator and parasite communities, and sexual selection. These factors often act in conjunction with each other, and therefore acoustic signals reflect the interplay among them (Forrest, 1994).

The vocalizations of birds have received considerable attention in the evolutionary context. Acoustic variation among regions in species that exhibit song-learning likely results from cultural

evolution (Catchpole and Slater, 2008), yet there is also evidence that acoustic differences are associated with biological evolution (MacDougall-Shackleton and MacDougall-Shackleton, 2001). Biological processes of song learning, dispersal, and gene flow are all expected to influence patterns of genetic and acoustic variation in birds. Approximately 80% of bird species are found at tropical latitudes, yet the majority of studies have been conducted on species that breed in the North Temeperate Zone (Podos and Warren, 2007; Stutchbury and Morton, 2008). As a consequence, the deficit of information on acoustic behaviour and acoustic structure in tropical birds is profound. Studies of tropical bird species are necessary given that tropical birds exhibit many different behaviours from temperate species, including differences in vocal behaviour (Slater and Mann, 2004; Langmore *et al.*, 2005). One key difference is that female song is common in many tropical bird species, but female song is rare or absent in species that breed in the North Temperate Zone. In this dissertation my motivation was to improve our knowledge on the songs of tropical birds and female birds by studying their vocal behaviour in detail. In particular, I focus on the evolutionary forces that underlie acoustic variation in tropical birds, and I compare acoustic variation between males and females to determine if the same evolutionary forces underlie song evolution in both sexes. Examining the forces that underlie song evolution in both sexes will improve our knowledge of animal communication generally, especially in those species where both males and females sing. In this General Introduction I provide background information for the five data chapters that comprise this dissertation, and introduce my study species, the Rufous-and-white Wren (*Thryophilus rufalbus*).

Cultural evolution and bird song

Several groups of animals learn to produce their vocalizations by emulating the vocalization of conspecifics, including birds, bats, primates, elephants, seals, and cetaceans (Janik and Slater, 1997; Jarvis, 2004; Poole *et al*., 2005; Sanvito *et al*., 2007). In birds, vocal learning has arisen in three

separate groups: hummingbirds (family: Trochilidae), parrots (order: Psittaciformes), and oscine songbirds (subfamily: Passeri; Jarvis, 2004). As a result, bird species serve as a model species in studies of culture and cultural evolution (Irwin, 2012). Song learning plays an important role in cultural evolution because copying errors (cultural mutations), cultural drift (changes in the composition and structure of songs in a population due to random processes), cultural selection, and migration can all give rise to new songs or change song frequencies within a population (Lynch, 1996).

Birds exhibit great acoustic diversity varying in vocal behaviour (e.g. how often they sing), vocal structure (e.g. the level of temporal and structural variation in their songs), and vocal complexity (e.g. the number of songs they are capable of singing; Catchpole and Slater, 2008). For example, whereas many birds produce only a single stereotyped song, many others are capable of producing two or more songs (referred to as a "song repertoire"; Catchpole and Slater, 2008). Although most of birds produce solo songs, others combine their songs temporally to produce duets (i.e. the coordinated songs of two individuals) and choruses (the coordinated songs of more than two individuals; Brown and Farabaugh, 1991; Seddon, 2002; Mennill and Vehrencamp, 2005; Hall, 2006; Mann *et* al., 2006; Tobias *et al*., 2016).

Local adaptation and acoustic signals

Habitat affects acoustic variation, and there is a large body of literature demonstrating the relationship between acoustic structure and habitat structure (Wiley and Richards 1978; Hunter and Krebs 1979; Boncoraglio and Sano, 2007). A central hypothesis explaining the relationship between acoustic structure and habitat is the Acoustic Adaptation Hypothesis (Morton, 1975). The Acoustic Adaptation Hypothesis states that signals are designed for optimal transmission through the natural environment of the animals that produce them; thus acoustic signals should be designed to maximize transmission and reduce degradation (Morton, 1975). Degradation is defined as the

changes that sounds experience as they propagate through the environment (Morton, 1986; Dabelsteen *et al*., 1993; Holland *et al*., 1998; Nemeth and Winkler, 2001). Degradation affects the amplitude, frequency composition, and temporal patterns of sounds through processes that include scattering, atmospheric turbulence, boundary effects, reverberation, and dispersion (Dabelsteen *et al.*, 1993; Badyaev and Leaf, 1997; Bradbury and Vehrencamp, 2011). Degradation is a greater problem in densely vegetated habitats (e.g. forests) because leaves, branches, and tree trunks can scatter or degrade signals (Richards and Wiley, 1980; Dabelsteen *et al.*, 1993; Badyaev and Leaf, 1997).

Although habitat affects acoustic signal design and transmission, there are other environmental factors that also influence animals' acoustic signals. For example ambient noise influences both the timing and structure of signals (Brumm and Slabbekoorn, 2005; Shannon *et al.,* 2015). In both urban and natural areas, animals alter the frequency of their signals to enhance long-range transmission in the presence of noise from traffic and abiotic sources (i.e noise from wind and rivers; Martens and Geduldig 1990; Slabbekoorn, 2004; Slabbekoorn and Peet, 2003; Hanna *et al*., 2011; Mockford *et al*., 2011). Additionally, noise from biotic sources (e.g. other animals) may influence animal signals. For example, some bird species alter the timing and frequency range of their in the presence of some insect and bird species to avoid having their signals overlapped (Luther, 2009; Tobias *et al*., 2014; Hart *et al*., 2015). All of these factors influence the signals of animals, and therefore animals should maximize signal transmission by producing signals that avoid interference from these potential noise sources (Shannon *et al.,* 2015).

Acoustic divergence and genetic divergence

Past studies have used a variety of both nuclear DNA and mitochondrial DNA markers to study genetic divergence (Toews and Breselford, 2012). Selectively neutral markers with high polymorphisms and mutation rates, such as DNA microsatellites (10^{-3}-10^{4}), are ideal for quantifying

and detecting contemporary genetic patterns and population structure (Jarne & Lagoda, 1996). Mitochondrial DNA (mtDNA) markers, in contrast, evolve at slower rates ($2x10^{-6}$; Garcia-Moreno, 2004) and can therefore provide greater insight into historical processes. These two markers also show contrasting patterns of inheritance, with DNA microsatellites being bi-parentally inherited and mtDNA being maternally inherited. Given these differences, mtDNA patterns will strictly reflect female dispersal and gene flow, whereas microsatellite patterns will reflect patterns of dispersal and gene flow in both males and females (Burg and Croxall, 2001). Given the different resolution that each marker provides, incorporating both nuclear and mtDNA markers will improve our ability to examine both contemporary and historical patterns genetic variation and gene flow.

Examining the factors that influence gene flow, contemporary population structure, and historical genetic patterns is a critical component of evolutionary studies. Historically, population genetic studies and phylogeography studies have examined genetic diversity and genetic variation in the context of species' geographic distributions to infer causes of current and historical population genetic structure (Richards *et al.,* 2007). More recent advances have focused on using a hypothesis-testing approach to investigate the evolutionary history of species (Carstens *et al*., 2005; Steel and Storfer, 2006; Richards *et al*., 2007), with the field moving towards a more integrative approach to examine the causal links between ecology, geography, selection, and the evolutionary history of individual species and communities (Hickerson *et al*., 2010). Just as this approach will help to further examine biological evolution, this approach can also be used to examine the evolutionary history of phenotypes and behaviours. Recent studies have used causal modeling and landscape genetic approaches to test the role of drift, ecology, geography, and selection on the evolution of phenotypic traits (Ruegg *et al*., 2006; Funke and Murphy, 2010; Wang and Summers, 2010). Using a hypothesis-testing approach will help to distinguish the factors that influence phenotypes, given

that a combination of environmental, genetic, and social factors often act together on the evolution of these traits (Richards *et al*., 2007; Funke and Murphy, 2010).

Comparisons are often made between biological and cultural evolution, given that similar forces drive both biological and cultural evolution (i.e. mutation, migration, drift, and selection; Lynch, 1996). Acoustic signals have been shown to co-vary with genetic variation, and carry a phylogenetic signal, furthering the idea that biological and cultural evolution are linked (Baker *et* al., 1982; McCracken and Sheldon, 1997; MacDougall-Shackleton and MacDougall-Shackleton, 2001; Price and Lanyon, 2004; Isler *et al*., 2005; Goicoecha *et al.*, 2010; Campbell *et al*., 2011). Although acoustic traits and genetic patterns may show similar patterns of variation, the two phenomena are not necessarily related to each other (Soha *et al*., 2004; Wright *et al*., 2005; Leader *et* al., 2008). Two key hypotheses have been proposed to explain the relationship between genetic and acoustic divergence. The Genetic Adaptation Hypotheses (Marler and Tamura, 1964) proposes that biological and cultural evolution are linked and that young birds learn songs in natal areas and select mates that sing local songs, resulting in a pattern where animals with different vocalizations are genetically distinct from one another. In contrast, the Drift Hypothesis (Andrew, 1962) proposes that biological and cultural evolution are not linked; populations may be genetically distinct, although similarities between the population acoustic structure and population genetic structure may arise as a result of reduced gene flow, habitat breaks or fragmentation, or historical patterns including long-term isolation, population bottlenecks, and founder effects (Moore *et al*., 2005, Dingle *et al*., 2008; 2010; Gonzalez *et* al., 2011; Parker *et al*., 2012; Caro *et al*., 2013; Sosa *et al.*, 2014). Given that acoustic and genetic patterns may arise independently of each other, pairing acoustic and genetic analyses together will help to determine the factors that influence acoustic variation. In particular, genetic analysis provides an important tool to quantify and measure gene flow, isolation, and genetic divergence to incorporate into the hypothesis-testing approach described above. This in turn will

help to determine the role that cultural drift, cultural selection, and environment have on the evolution of acoustic signals.

Behavioural differences between sexes

Although bird song has played an integral role in the field of bioacoustics, most research has focused on male song, particularly on the songs of male birds living in the North Temperate Zone (Reibel *et* al., 2005). Fewer studies have focused on the songs of female birds, because female songs are less common in this region (Slater and Mann, 2004; but see Garamszegi *et al*., 2007), in spite of the fact that female song is ancestral in birds (Odom *et* al., 2014). In contrast to the pattern in the North Temperate Zone, female song is prevalent in tropical environments. In the tropics, it is commonplace for both sexes to sing, although males and females often vary in vocal output, repertoire size, and acoustic structure (e.g. Brown and Farabaugh, 1991; Mennill and Rogers, 2006; Hall *et al*., 2015). Acoustic differences between the sexes suggest that different evolutionary forces may act on the signals of males and females (Mennill and Rogers, 2006), although few studies have focused on the forces that drive the evolution of female song (Garamszegi *et al*., 2007; Price, 2015).

Similar to sex differences in vocal behaviour, male and female birds often exhibit different dispersal behaviours. In birds, dispersal is often female-biased, with females dispersing more often and farther than male birds (Greenwood, 1980; Greenwood and Harvey, 1982; Clarke *et al.*, 1997; Wolffe *et al*., 1998). Given that males and females often display different dispersal strategies, this allows us to compare the role of dispersal in cultural evolution (Lynch, 1996). Dispersal is thought to decrease acoustic variation between populations, and increase cultural diversity within populations, because immigrants may introduce new or unique syllables and songs from other populations (Stewart and MacDougall-Shackleton, 2008; Fayet *et al*., 2014).

Study system

Primarily found in the new world, there are currently 85 species of birds in 19 genera recognized in the family Troglodidae (Wrens; Kroodsma and Brewer, 2005; Lara *et al*., 2012). Although wrens are often overlooked because of their plain appearance, members of this family are renowned for their vocal capabilities, complexity, and diversity (Brewer, 2001). Many wren species exhibit female song, as well as vocal duets (where males and females combine their songs to produce coordinated songs; Levin, 1996; Mennill and Vehrencamp, 2005; Logue 2006; Mann *et* al., 2006; Templeton *et al*., 2011). Given that female song is commonly found in this family, wrens are an ideal study system to examine the ecology and evolution of female song, and compare patterns between sexes.

Rufous-and-white Wrens (*Thryophilus rufalbus*) are non-migratory songbirds that inhabit tropical forest habitats, from Southern Mexico through Central America, and into Colombia and Venezuela in South America (Figure 1.1; Stotz *et al.,* 1997). Males and females are similar in appearance, although females tend to be smaller (Valderamma *et al*., 2007). Both males and females produce songs, and songs are structurally different between sexes, with females typically producing songs with fewer notes, shorter trills, and at higher frequencies than males (Mennill and Vehrencamp, 2005). Males and females both possess song repertoires, singing up to 15 different song types, although repertoires are larger in males than females (Brenowitz and Arnold, 1986; Mennill and Vehrencamp, 2005; Harris *et al*., 2016). Given that males and females both produce solo songs, this system is ideal for making direct comparisons of acoustic variation between sexes.

Thesis goals and objectives

The primary goal of my dissertation is to compare patterns of cultural evolution in male and female Rufous-and-white Wrens, and determine the evolutionary forces that act on male and female acoustic signals. I combine acoustic analysis with molecular genetic analysis to examine the

influence of genetic and cultural drift, immigration, selection, and ecology have on local patterns of acoustic variation (i.e. variation within a single population) and broad-scale patterns of acoustic variation (i.e. variation within and between multiple populations). Using this integrative approach I address the following questions: Do male and female Rufous-and-white Wrens exhibit similar patterns of acoustic variation? Do acoustic differences reflect ecological, genetic, or cultural selection differences between populations? Do patterns of acoustic variation relate to between-sex differences in dispersal and song learning strategies?

My dissertation includes five data chapters. In Chapter 2 I present a sound transmission experiment in three populations of Rufous-and-white Wrens to test the effect of ecological variation on song evolution, and evaluate whether male and female songs are adapted to their local environments. In Chapter 3, I compare patterns of acoustic variation between multiple populations, and determine the role that acoustic adaptation, cultural drift, genetic drift, and historical isolation play in driving geographical patterns of acoustic variation. In Chapter 4, I quantify the effect of immigration on acoustic variation, and I test whether first-generation migrants introduce new songs from their natal populations into their breeding populations. In Chapter 5, I use an 11-year dataset to compare patterns of acoustic and genetic change, and I examine the influence of cultural drift and genetic drift on acoustic variation. Finally, in Chapter 6, I use this long-term dataset to examine whether dispersal is sex-biased, and the effect that dispersal has on spatial acoustic structure and spatial genetic structure in Rufous-and-white Wrens.

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Figures

Figure 1.1: Top: Map of Central America and northwestern part of South America showing the distribution range of Rufous-and-white Wrens (*Thryophilus rufalbus*) in green (Birldife International and NatureServe, 2015). Bottom: Rufous-and-white Wrens.

Chapter 2: A test of the Acoustic Adaptation Hypothesis in three types of tropical forest: degradation of male and female Rufous-and-white Wren songs*

*This chapter is the outcome of joint work with L. Sandoval, T. Dabelsteen, and D. Mennill

Chapter Summary

Many animals produce complex vocalizations that show pronounced variation between populations. The Acoustic Adaptation Hypothesis helps to explain this variation, suggesting that acoustic signals are optimized for transmission through different environments. Little is known about the transmission properties of female vocalizations because most studies of the Acoustic Adaptation Hypothesis have focused on male vocalizations of organisms living at temperate latitudes. We explored the relationship between environmental variation and the transmission properties of songs of Rufous-and-white Wrens, resident Neotropical songbirds where both sexes sing. Using playback, we broadcast and re-recorded elements of male and female songs from three populations of wrens living in three different forest habitats in Costa Rica. We measured four variables of the re-recorded sounds: signal-to-noise ratio, excess attenuation, tail-to-signal ratio, and blur ratio. Our results show a significant difference between transmission characteristics of both male and female song elements across the three habitats, indicating that sounds transmit differently through different types of tropical forest. The population from which the broadcast sounds were recorded (source population) had little effect on sound transmission however, suggesting that acoustic differences between these populations may not arise through acoustic adaptation to these habitats. Male and female elements showed similar transmission properties overall, although signal-to-noise ratio of male elements was influenced by source population, whereas blur ratio and excess attenuation of female elements were influenced by source population. Our study highlights the differences in transmission characteristics of animal sounds through different habitats, and reveals some sex differences in transmission properties.

Introduction

Diverse animal taxa produce long-range acoustic signals that play an important role in mate attraction and resource defense (Bradbury and Vehrencamp, 2011). Animal acoustic signals exhibit incredible diversity, and many signals vary geographically (Marler and Tamura, 1962; Irwin *et al.,* 2001; Campbell *et al.,* 2010; Trefry and Hik, 2010). Geographic variation in acoustic signals can play an important role during speciation when different populations develop divergent acoustic signals and then fail to recognize each other following secondary contact (Irwin *et al*., 2001). Given the role that acoustic divergence can play in evolution, understanding the forces that drive acoustic divergence remains an important area of research (Wilkins *et al.,* 2013).

Habitat affects the evolution of acoustic signals (Morton 1975; Wiley and Richards 1978; Hunter and Krebs, 1979; Handford and Lougheed, 1991; Dabelsteen *et al*., 1993; Boncoraglio and Saino, 2007). This widely-supported fact lead Morton (1975) to propose the Acoustic Adaptation Hypothesis: acoustic signals are optimized for transmission through the natural environment of the animals that produce them, and acoustic signals used for long-range communication should exhibit adaptations that minimize degradation and maximize transmission (Morton, 1975; Marten *et al.,* 1977; Boncoraglio and Saino, 2007). A review by Boncoraglio and Saino (2007) found that song characteristics of forest and non-forest birds vary between habitats, providing further support that the Acoustic Adaptation Hypothesis may explain acoustic divergence between and within species (Hunter and Krebs, 1979; Tubaro and Segura, 1994; Slabbekoorn and Smith, 2002b). Other studies have found less support for the Acoustic Adaptation Hypothesis (Rothstein and Fleischer, 1987; Date and Lemon, 1995; Daniel and Blumstein 1998; Doutrelant *et al.*, 1999; Trefry and Hik, 2010), although a failure to find a relationship between habitat and acoustic characteristics does not mean that habitat does not affect animals' acoustic signals (Barker, 2008). In addition to habitat, many other factors influence the evolution of acoustic signals, including morphology, phylogeny,

physiology, sexual selection, social eavesdropping, predators, learning, founder effects, drift, and other aspects of the environment (e. g. humidity and ambient noise; Forrest, 1994; Lynch, 1996). These factors may act in concert and therefore the evolution of acoustic signals is necessarily complex, and likely to reflect interactions among these various factors (Forrest, 1994; Wilkins *et al.,* 2013).

Dense vegetation can cause significant problems for the transmission of acoustic signals (Bradbury and Vehrencamp, 2011). In particular, leaves, branches, and tree trunks can degrade signals, changing sounds as they propagate through the environment (Richards and Wiley, 1980; Badyaev and Leaf, 1997; Dabelsteen *et al.,* 1993). Degradation is expected to affect amplitude, frequency composition, and temporal patterns of sounds through processes that include scattering, atmospheric turbulence, boundary affects, reverberation, and dispersion (Richards and Wiley, 1980; Dabelsteen *et al.*, 1993; Bradbury and Vehrencamp, 2011). Given the important role that habitat plays on the evolution of songs, testing the transmission properties of an animal's acoustic signal through its environment will provide further insight into some of the constraints that affect the evolution of signals.

Tropical species present exciting systems for studying the effects of habitat on acoustic signals, given the high diversity of habitat types, and the dramatic differences in habitats over relatively short distances (Stutchbury and Morton, 2001). Population-level studies of broadly distributed species are especially revealing, because they provide the opportunity to examine characteristics of acoustic signals in animals that inhabit a diverse range of habitats (e.g. Handford and Lougheed, 1991; Slabbekoorn and Smith, 2002). The high rates of philopatry and heightened habitat specialization that are common to many tropical bird species (Stutchbury and Morton, 2008) suggest that tropical animals may be locally adapted to their habitats. Yet most studies of the Acoustic Adaptation Hypothesis have been conducted on temperate species, and focus on male

song (Barker, 2008). Tropical bird species are interesting from an acoustic perspective, given that females of many tropical bird species sing (Slater and Mann, 2004), an uncommon phenomenon in north-temperate animals (Price *et al.,* 2009). Studying the acoustic signals of female birds is important (Barker, 2008), given that female song is an ancestral trait in birds (Odom *et al.,* 2014), and many aspects of female song production and development remain poorly understood (Reibel, 2003). Comparisons of male and female song characteristics offer a compelling area of research given that very few geographic-level comparisons have been made between male and female song characteristics (but see Mennill and Rogers, 2006).

To investigate acoustic adaptation across both sexes and among different types of tropical habitats, we studied the transmission properties of songs of Rufous-and-white Wrens (*Thryophilus rufalbus*), a year-round resident of Central America and northwestern South America. This species lives in a variety of forested habitats across its range (Stiles and Skutch, 1989; Stotz *et al.,* 1996). Interestingly, both male and female Rufous-and-white Wrens sing solo songs and produce coordinated duets by combining their solo songs (Mennill and Vehrencamp, 2005). Both males and females possess song repertoires, singing up to 15 different song types (Harris *et al*., 2016), although male repertoires are larger than female repertoires (Mennill and Vehrencamp 2005). Male and female songs include similar characteristics, beginning with varied introductory elements, followed by a trill (the longest part of the song), and usually concluding with a single loud note that is often the highest frequency part of the song (Mennill and Vehrencamp 2005). Given that both sexes sing within this species, this system allows us to compare patterns between sexes and further our understanding of female song.

We used recordings of played-back songs to examine the transmission properties of both male and female Rufous-and-white Wren songs in three different populations in Costa Rica. Previous work has demonstrated that songs of Rufous-and-white Wrens vary geographically (Valderrama *et*

al., 2007), and our ongoing research confirms that songs are variable among our three study populations (based on fine structural measurements, i.e. syllable length, bandwidth and dominant frequency of the trills). Our three study sites vary in habitat structure, vegetation density, and climate (Clark *et al.,* 2002; Mata and Echeverria, 2004), and therefore acoustic differences may reflect local adaptations at each site. We sought to test whether variation between songs among populations shows evidence of acoustic adaptation. Specifically, we explored the relationship between habitat and acoustic structure of male and female Rufous-and-white Wren songs, testing whether sound propagation varied with playback site (i.e. the location where sounds were broadcast and re-recorded) and source population (i.e. the location where the stimuli were recorded).

Methods

Study Site

We conducted our experiment at three sites in Costa Rica: Sector Santa Rosa of the Guanacaste Conservation Area (10.8836 °N, 85.7750 °W, 300m a.s.l.); Sector Rincón de la Vieja of the Guanacaste Conservation Area (10.8300 °N, 85.3239 °W, 1000m a.s.l.); and the San Luis Valley of Monteverde at the University of Georgia Costa Rica field site (10.2380 °N, 84.7970 °W, 1100m a.s.l.). Populations of free-living Rufous-and-white Wrens are found at all three sites. Playback sessions took place in 2013 on April 17-18 at San Luis, June 1-2 at Rincon de la Vieja, and June 11-12 at Santa Rosa during the onset of the breeding season at each population (birds breed earlier at San Luis than the other two sites; pers. obs.). All playback sessions were conducted between 0700 and 1100 h, when this species is most vocally active (Mennill and Vehrencamp, 2005). We conducted our experiment over a two-day period at each site, to ensure that weather conditions like temperature, relative humidity, and wind were consistent throughout the experiment. Daily temperatures were consistent with mean monthly values at each of the sites (average temperature and relative

humidity ranged from 23.0 °C and 72.6% at the montane forest site, 26.0°C and 84.0% at the wet site and 27.3 °C and 76.0% at the dry forest site over the two day periods), and therefore we feel confident that the meteorological conditions are representative of conditions at each site during the appropriate time of year.

Our three study sites differ in both vegetation and precipitation (Clark et al., 2002; Mata and Echeverria, 2004). (1) Santa Rosa (hereafter referred to as the "dry forest" site) is a tropical dry forest (following the Holdridge Life Zone classification system, Holdridge, 1967) with a dry season that lasts from November to April and an intense rainy season from May to November (1876 mm on average/year from 1998 to 2013; NASA TRMM project). The understory at this dry forest site is relatively open (basal area = 25.0 $m^2 Ha^{-1}$ for stems >10 cm; Gillespie *et al.,* 2000) especially during the dry season, when the majority of shrubs in the understory are leafless. Vegetation density increases following the start of the rainy season. The canopy attains heights of approximately 20m although some emergent trees reach heights of 30m (Janzen, 1983). (2) Rincon de la Vieja (hereafter referred to as the "wet forest" site) is a Premontane Moist-Wet Forest (Holdridge, 1967), with a dry season from January to April (2057 mm average/year from 1998 to 2013; NASA TRMM project). This area is wetter than the lowland dry forest, but receives less precipitation than forests at higher elevations. This forest type is representative of many mid-elevation forests (~900m elevation); the understory is relatively open, with fewer shrubs found here than in the dry forest (basal area = 31.2$m^2 Ha^{-1}$; Heaney and Proctor, 1990; basal area data are not available from our wet forest site, and this value is chosen for a comparison site in Costa Rica with similar vegetation, climate, and altitude). The canopy attains heights of 25-30m, and many large trees, including figs, dominate the forest (Janzen, 1983). (3) San Luis field station at Monteverde (hereafter referred to as the "montane forest" site) is a Lower Montane Wet Forest (~1100 m elevation; Holdridge, 1967), with a season of less precipitation lasting from January to April. This area receives greater precipitation

than our other two sites (2706 mm average/year from 1998 to 2013; NASA TRMM project). The understory is densely vegetated by shrubs, ferns, and palms (basal area = 62.0 m^2 Ha^{-1} for stems >10 cm; Nadkarni *et al.,* 1995) with epiphytes covering 50-70% of the tree trunks. Consequently, this habitat is much more dense than the understory at our other two sites (Janzen 1983). The canopy at the montane forest site reaches heights of 25-30 m, dominated by diverse large tree species.

Song type selection

For our playback stimuli we used both male and female songs that we recorded from each of the three study populations in 2012. Recordings were collected using a solid-state digital recorder (PMD-660 Marantz; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70). To create our stimuli we chose five of our highest-quality songs from each population for each sex (each song used for the stimuli came from a different individual), using only songs with high signal-to-noise ratio (assessed visually based on sound spectrograms) and no overlap from other conspecific or heterospecific sounds. From those songs, we selected population-specific elements that were representative of elements that were most common in each population during our recording sessions. To create our final playback stimuli, we selected 18 male song elements (six from each population, giving rise to six introductory, trill, and terminal syllables overall; Figure 2.1) and 20 female song elements (six from the montane and dry forest sites and eight from the wet forest site, giving rise to seven introductory, and terminal syllables, and six trill syllables, overall; we included two additional elements for wet forest females to reflect the diversity of female song elements in that population; Figure 2.1). We determined that six elements from each sex at each population was an appropriate number, given that the elements we selected for both male and female playback are widespread and frequently used within each population. Our sample size (n=18 elements for males and n=20 elements for females) is comparable to previous transmission studies of species with intermediate to large song repertoires (Holland *et al.,* 1998;

Barker *et al.,* 2009; Mockford *et al.,* 2011). We isolated and filtered songs and elements using the "FFT filter" function of Audition software (version 3.0, Adobe Systems, San Jose, CA, USA); for each sound, we used a different filter (see page 1 of supplementary material for information on the filters used to isolate each sound), given that each sound occupied a different bandwidth.

We focused our analysis on elements within the male and female songs, rather than entire songs, because we were interested in understanding how the degradation of single elements contribute to the degradation of entire songs. Examining elements separately from entire songs is important, given that the context in which sounds are broadcast can affect the acoustic properties; for example reverberation is known to enhance both the length and amplitude of a sound, especially for the pure tone elements used by many forest birds, that change little in frequency (Slabbekoorn *et al.,* 2002; Nemeth *et al.,* 2006). While we present the results for elements only in this manuscript, we did analyze entire songs in another analysis, and we found that songs showed a similar pattern to elements (see supplementary material Tables 2.S3-2.S7).

Using these prepared sounds, we created playback tracks by pasting the stimuli into a single file using Audition (Adobe Systems Inc., San Jose, CA). Each stimulus track included 5.0 s of silence at the outset (facilitating a measurement of background noise), followed by each of the sounds in succession, with 1.5 s of silence between each sound (preventing sounds from being overlapped by the end of the previous sound). Each playback stimulus was played five times in succession to maximize the chances of recording multiple examples of each element without overlap from background sounds. Each repetition was separated by 5.0 s of silence before the next repetition began.

Experimental setup

At each of our three sites, we conducted our transmission experiment in three different Rufous-and-white Wren territories. We chose territories that were representative of the common

vegetation at each site. Within each territory we positioned both the speaker and microphone at a single height above ground (1.5 m). This height falls within the range of perch heights (1 to 5 m) male and female Rufous-and-white Wrens are most commonly observed using as song posts (Barker and Mennill, 2009). We placed the microphone at four separate distances (5m, 10m, 20m and 40m) from the speaker. We chose 20 m as one important distance based on a previous microphone array study that found 20 m to be the average distance separating male and female Rufous-and-white Wrens while performing duets (Mennill and Vehrencamp, 2008). The maximum (40m) and minimum (5 and 10 m) distances were chosen based on doubling and halving this average distance. Unlike previous studies (e.g. Barker et al., 2009; Sabatini *et al.*, 2011), where playback was conducted along a linear transect, we distributed the four distances at different axes within each territory (as in Sandoval *et al.,* 2015). We employed this approach rather than a linear transect design to avoided sampling the same sections of habitat across the four distances tested. Using a linear transect design would result in the same section of habitat being included multiple times (e. g. the first 5m is sampled at all four distances), and therefore the subsequent three distances are not independent of the first 5 m transect, while the 40 m trial would not be independent of the first three transects. Furthermore by using this approach, we attempted to include more of the birds' territories in our transmission tests, thus providing a more representative sampling of the effect of habitat on sound transmission. We chose these playback axes according to the cardinal points in all of the nine territories where we conducted our playback.

We broadcast sounds using an active loudspeaker (Anchor Audio, Minivox; frequency response 0.1-12 KHz), and re-recorded them using an omnidirectional microphone (Sennheiser ME62) and a solid-state recorder (PMD-660 Marantz; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format), connected to a pre-amplifier (Sound Device MP-1: Frequency Response 0.02-22 KHz). Playback was broadcast at 75 dB (as measured at 1m distance using a sound meter; Radio Shack

model 33-2055 using C-weighting slow response), allowing us to match the sound pressure level that has been used in a previous study of Rufous-and-white Wrens songs (Barker *et al*., 2009). We increased the gain on our pre-amplifier to 18 dB and 28 dB for the 20 m and 40m trials respectively, and we corrected for these changes in gain by adding 18 and 28 db to the appropriate analyses. Changing the gain was critical in these recordings, because the same recording levels could not be used to collect high-quality recordings for both the short and long transmission distances.

Sound Analyses

As in most other transmission studies (e.g. Holland *et al.,* 1998; Lampe *et al.,* 2007; Barker *et al.,* 2009), we used SigPro software (v 3.25; Pedersen, 1998) to analyze the transmission properties of all recorded sounds. We compared recorded sounds at the four distances (5, 10, 20 and 40 m) against a model signal. The model signal used for comparison was obtained by broadcasting our male and female stimuli with the aforementioned playback and recording apparatuses, but with a separation distance of just 1.25 m at a height of 1.5 m on a flat dirt road in Sector Santa Rosa—i.e. an environment with no vegetation (in a 20m radius) that could influence the transmission between the speaker and the microphone—on a calm morning with little or no background noise (e.g. wind). We then filtered and trimmed these recordings for the purpose of removing any potential tails or echoes introduced during the model signal recording. We used these model signals, rather than the original stimuli, to account for any noise that might have been introduced by the playback or recording equipment (as in Lampe *et al.*, 2007, for example).

We compared degraded sounds to model sounds to obtain four measurements of degradation (for details see Dabelsteen *et al.,* 1993 and Holland et al., 2001): signal-to-noise ratio, tail-to-signal ratio, blur ratio, and excess attenuation. We also measured background noise by sampling the background sound immediately prior to each stimulus recording (as described by Dabelsteen *et al.,* 1993). We assumed that this background sound matched the noise overlapping

our re-recorded playback sounds (Holland *et al.,* 1998; Barker *et al.,* 2009; Sabatini *et al.,* 2011,). Background noise was filtered within the same frequency ranges as the test sounds and then used to calculate signal-to-noise ratio and better understand how signal-to-noise ratio varied among our three forested sites, as described in Dabelsteen *et* al. (1993. Thus we measured and compared background noise at each area, so that we could quantify the level of environmental noise at each site for each sound within its frequency range, past studies have shown that the background noise varies with frequency, that there are differences in the amount of ambient noise between forested habitats, and that these differences can affect sound degradation (Slabbekoorn *et al.,* 2002).

For each sound we analyzed up to three re-recorded exemplars per distance along each transect, although in some instances we were unable to measure all three due to overlap by background noise. Due to windy conditions at Monteverde, we were only able to collect useful measurements for two of the three transects at 5, 10 and 20 m and only one of the three transects at 40m; the remaining sounds were too heavily overlapped by background noise. After omitting these overlapped sounds, we were left with 1600 measurements for male song elements (2.47±1.00 per distance in each transect; mean± SE), and 1770 for female song elements (2.46±1.01).

Statistical Analyses

To analyze degradation of Rufous-and-white Wren sounds, we used linear mixed models. We analyzed the sexes independently with separate models. We analyzed sexes separately in this chapter (as well as throughout this dissertation) because males and females exhibit structural differences with respect to their acoustic signals (Mennill and Vehrencamp, 2005). Furthermore, males and females exhibit differences in vocal output and signing behaviour (Topp and Mennill, 2008; Barker and Mennill, 2009, and therefore we thought it more appropriate to examine sexes independently because of the pronounced acoustic differences between males and females. We used the four sound degradation measurements (signal-to-noise ratio, blur ratio, tail-to-signal ratio,

and excess attenuation) as our response variables and ran each of the measurements in a model for each of the sexes (i.e. eight models in total). For each model we had four independent variables: playback site (three levels corresponding to the three sites where we conducted playback), source population (three levels corresponding to the three populations where birds were recorded), distance (four levels, corresponding to the four distances between loudspeaker and microphone), and element type (three levels, because we were interested in seeing if there were differences in the degradation of introductory, trill, and terminal elements; Mennill and Vehrencamp 2005). For our analysis we examined main effects and two-way interactions for each model. We used Tukey post-hoc tests to evaluate whether differences in means were significant. To analyze background noise (dB) during the transmission experiments, we ran two additional models, one for each sex. Like our models for sound degradation we had four independent variables (playback site, source population, element type and distance), but for our background noise analysis we examined only main effects.

To understand whether Rufous-and-white Wrens' song elements show local adaptation to the environment where the birds are found, we focused on the interaction playback site × source population. We focused specifically on this interaction, based on our expectation that elements that are adapted to their local environment should transmit more effectively (i.e. experience less degradation) at the playback site where they were originally recorded.

We report all values as mean ± SE. All analyses were performed in JMP (version 10.0; SAS Institute, Cary, NC, U.S.A.).

Results

Our transmission data reveal that playback site and source population had different effects on the degradation of male and female Rufous-and-white Wren song elements; transmission properties regularly showed a significant effect of playback site, but rarely showed a significant effect of source

population. Below, we present detailed findings for male and then female song elements, describing the main effects followed by the interaction terms.

Males

For male song elements, signal-to-noise ratio, tail-to-signal ratio, and excess attenuation were all significantly affected by playback site (Table 2.1); signal-to-noise ratio was higher at the wet and dry forest sites than at the montane forest site, tail-to-signal ratio was higher at the dry forest site than the other two sites, and excess attenuation was greater at both the wet and dry forest sites than the montane forest site (Figure 2.2). Signal-to-noise ratio was the only measurement that was significantly affected by source population (Table 2.1); elements recorded from the montane and wet forest sites had a higher signal-to-noise ratio than elements recorded from the dry forest site (Figure 2.3). All four sound degradation measurements were significantly affected by distance (Table 2.1); degradation increased as distance from the speaker increased (Table 2.S1). Three of the four sound degradation measurements (signal-to-noise ratio, blur ratio, and excess attenuation) showed significant variation with element type (Table 2.1); signal-to-noise ratio was higher for introductory and terminal elements than trill elements, blur ratio was higher for terminal elements than either introductory or trill elements, and excess attenuation was higher for introductory elements, than either terminal or trill elements (Figure 2.4).

All four sound degradation measurements showed significant interaction effects in our analysis of male song elements, especially for the interactions between playback site × distance (Table 2.S1) and source population × element type (Table 2.1). Signal-to-noise ratio of elements for the interaction playback site × distance was significantly higher at shorter distances (both 5 and 10m) at the wet and dry forest sites, and lowest at the furthest distances (20 and 40m) at the montane forest site (Table 2.S1). Like the patterns observed for signal-to-noise ratio, tail-to-signal and blur ratio were higher for elements at the furthest distances at all three sites, while excess

attenuation was greatest at the furthest distances at the wet and dry forest sites, with the lowest values at the shortest distances at the montane forest site. For the interaction between source population × element type, most element types recorded from both montane and wet forest sites had a higher signal-to-noise ratio than element types recorded from our dry forest site (Table 2.S2). Tail-to-signal ratio was lower for terminal and introductory elements from the montane and wet forest sites, while tail-to-signal ratio was highest for trill elements recorded from the montane and wet forest sites and introductory and terminal elements recorded from the dry forest site. Wet forest terminal and trill elements along with dry forest terminal elements showed a lower blur ratio than dry forest trill elements. Finally, excess attenuation was significantly higher for montane and wet forest introductory elements than trill or terminal elements from the same populations (Table 2.S2). Only a single interaction (signal-to-noise ratio) was significant for the interaction playback site × source population; however sounds did not show significantly less degradation at the sites where they were recorded (i.e. the degradation of elements recorded at the dry forest was not lower than elements recorded at our wet and montane forest sites, when played at our dry forest site; Figure 2.5). Elements recorded from montane and wet forest sites had a higher signal-to-noise ratio at the wet and dry forest sites, while signal-to-noise ratio of elements (from all three populations) played at the montane forest site had the lowest signal-to-noise ratio values. Signal-to-noise ratio was the only variable to show a significant relationship for the interaction between playback site × element type, where introductory and trill elements played at the montane forest site had the lowest signal-to-noise ratio values from all others (Table 2.S1). There were no significant effects for the interaction source population × distance, while distance × element type affected signal-to-noise ratio and blur ratio only. For signal-to-noise ratio, elements at the closest distances (5m) had a higher signal-to-noise ratio than elements at the farthest distances (40m). Meanwhile terminal elements at farther distances (20 and 40 m) had a significantly higher blur ratio than all other

element types, while trill and introductory elements at shorter distances (5 and 10m) had the lowest blur ratio values (Table 2.S1).

Females

For female song elements, sound degradation was significantly affected by most of the main effects (Table 2.2). Signal-to-noise ratio, tail-to-signal ratio, blur ratio, and excess attenuation were all significantly affected by playback site. Female elements showed a higher signal-to-noise ratio and tail-to-signal ratio, lower blur ratio, and experienced greater excess attenuation at the dry forest site, while elements played at the montane forest site exhibited a lower signal-to-noise ratio and tail-to-signal ratio, higher blur ratio, but experienced less excess attenuation (Figure 2.2). Source population affected tail-to-signal ratio, blur ratio, and excess attenuation (Table 2.1). While post-hoc tests revealed no differences among sites for tail-to-signal ratio, female elements recorded from the montane forest site had a lower blur ratio than elements from the other two populations; elements recorded from the dry and montane forest showed greater excess attenuation than elements recorded from the wet forest (Figure 2.3). Like male elements, all four measurements were affected by distance, and elements showed greater degradation at the furthest distances (Table 2.S2). Lastly three of the four measurements (signal-to-noise ratio, tail-to-signal ratio, and blur ratio) were affected by element type (Table 2.2), and terminal elements had a higher signal-to-noise ratio, higher tail-to-signal ratio and higher blur ratio than both introductory and trill elements (Figure 2.4).

Half of the interactions showed significant effects in our analysis of female song elements (Table 2.2). Signal-to-noise ratio was the only measurement that showed a significant pattern for playback site × source population, where elements had a significantly higher signal-to-noise ratio when played at our dry forest site than at our montane and wet forest sites, and elements played at wet forest site had a significantly higher signal-to-noise ratio than elements at our montane forest site (Figure 2.5). However, as we observed for males, degradation of non-local elements was not

significantly greater than that of local elements outside of the populations where they were recorded. All four degradation measurements were significant for the interaction playback site × distance (Table 2.S2); song elements experienced significantly greater degradation as distance from the speaker increased, similar to patterns observed for males. Signal-to-noise ratio was significant for playback site × element type (Table 2.S2), and elements had a significantly higher signal-to-noise ratio at the dry forest site, followed by the wet and montane forest sites (Table 2.S2). Only blur ratio showed a significant effect for the interaction between source population × distance; elements from the wet and dry forest sites at the furthest distances had a higher blur ratio than elements from the montane forest site at all distances (Table 2.S2). Signal-to-noise ratio, tail-to-signal ratio, and blur ratio were significant for source population × element type, where signal-to-noise ratio was significantly lower for trill elements from all populations than the majority of terminal and introductory elements (Table 2.S2). Tail-to-signal ratio was lower for terminal elements recorded from our montane forest site, while introductory elements from our montane forest site have the longest tails. Greater blur ratio was exhibited by terminal elements from the dry and wet forest sites than introductory and trill elements (Table 2.S2). Finally blur ratio was the only measurement significant for element type x distance, and revealed that terminal and introductory elements at the furthest distances (20 and 40m) experienced a higher blur ratio than trill elements at all distances (Table 2.S2).

Background Noise

Transmission experiments for both male and female song elements showed that background noise varied by site (Table 2.3). Background noise at the montane forest site was significantly higher than at the wet and dry forest sites, which were not significantly different from one another (Table 2.S7). Source population did not show a significant effect for background noise levels for either male or female elements (Table 2.3), while distance significantly affected both male

and female songs, where background noise increased with distance between the loudspeaker and the microphone (Table 2.S7).

Discussion

Using a sound-transmission experiment, we tested the influence of habitat on the transmission of male and female Rufous-and-white Wren song elements in three different types of tropical forest, thereby testing predictions of the Acoustic Adaptation Hypothesis. We found that playback site affects the transmission of both male and female elements, and significant differences in background noise levels among sites. Source population (i.e. the location where songs were recorded) had little effect on degradation, given that only four of eight degradation measurements were significant (i.e. signal-to-noise ratio for male elements and tail-to-signal ratio, blur ratio, and excess attenuation for female elements). Furthermore the interaction playback site × source population did not suggest that song elements are locally adapted, given that elements did not experience less degradation at their respective sites (for example, dry forest song elements did not experience less degradation at the dry forest site in comparison to elements recorded at our wet or montane forest sites; Figure 2.5). Overall, Rufous-and-white wren songs appear to be optimized for transmission through forested habitat in comparison to open habitats (Barker *et al.,* 2009), but our data reveal that their song elements are not specifically adapted for transmission through different types of tropical forests. We conclude that habitat influences sound transmission of both male and female songs, but that sounds in these three study populations do not show strong evidence of acoustic adaptation to the three different habitats.

Playback Site

The Acoustic Adaptation Hypothesis predicts that the signals of animals living in densely vegetated habitats should be adapted for transmission through these habitats (Richards and Wiley, 1980; Badyaev and Leaf, 1997). Support for the Acoustic Adaptation Hypothesis is mixed (Ey and

Fisher, 2009); many studies have demonstrated support for the hypothesis (Hunter and Krebs 1979; Tubaro and Tugaro, 1994; Perla and Slobodchikoff, 2002; Van Dongen and Mulder, 2006; Derryberry, 2009), whereas other studies have failed to show support (Rothstein and Fleischer, 1987; Date and Lemon, 1995; Daniel and Blumstein, 1998; Doutrelant *et al.,* 1999; Trefry and Hik, 2010). We found that playback site had a significant effect on the degradation of both male and female acoustic signals. Environmental differences such as vegetation density, atmospheric absorption, and ambient noise all affect sound transmission (Brumm and Naguib, 2009), and differences in these factors between our three sites surely played a role in the transmission properties we described. We observed greater degradation at the montane and wet forest sites than at the dry forest site with regards to tail-to-signal ratio and blur ratio of both male and female elements. Vegetation density and rainfall are higher at the montane and wet forest sites than the dry forest site, where the habitat is more open (Nadkarni *et al.,* 1995; Gillespie *et al.,* 2000). Densely forested habitats result in greater degradation because there are more leaves, stems, branches, and trunks, thereby increasing the effect of reflection, refraction, and diffraction on sound waves (Naguib 2003).

In contrast to the pattern for tail-to-signal ratio and blur ratio, excess attenuation was significantly lower at the montane forest site than at the wet and dry forest sites, for both male and female elements. While vegetation density does affect excess attenuation, other factors such as atmospheric scattering and turbulence, as well as boundary interference, also affect attenuation (Brumm and Naguib, 2009). Humidity and temperature are known to affect the attenuation of sounds, and sounds experience less attenuation in humid air and when temperatures are cooler (Ingård, 1953; Griffin, 1971). Among the three study sites, the montane forest site receives the highest annual rainfall; humidity is greater (an average of 91% throughout the year; Johnson *et al.,* 2005) and temperatures are cooler (mean = 20.7°C; www.worldclim.org) than at the other two sites

(by comparison the average humidity in the dry forest ranges from 20-60% during the dry season and temperatures are warmer; mean = 24.8°C; Janzen, 1988; Clark *et al*., 2002; Mata and Echeverria, 2004). Therefore, climate differences among sites may contribute to the differences in excess attenuation we observed, as has been suggested in previous studies (Morton, 1975; Nottebohm, 1975), although we are aware of no studies that have tested the effect of climate differences between sites on sound transmission.

Signal-to-noise ratio of both male and female elements was significantly higher when sounds were played at the wet and dry forest sites than at the montane forest site. These differences may be attributable to the much noisier environment at our montane forest site, an idea that was directly supported by our comparisons of background noise (Table S7). Conditions at the montane forest site were much windier than at the other two sites and wind produces low frequency noise in the range of 0.1-1.0 KHz (Bradbury and Vehrencamp, 2011). The added background noise masked some of the elements used for playback during our experiment, especially those produced around 1.0 KHz (e.g. the introductory and trill elements of many male and some female songs are produced at this frequency; Mennill and Vehrencamp, 2005). Additionally, within highland tropical forests there is considerable background noise in the high frequency spectrum (Ryan and Brenowitz, 1985; Slabbekoorn and Smith, 2002). Animals like cicadas call continuously, with this noise band beginning around 2 KHz and extending up to 5 KHz (Slabbekoorn, 2004). A recent study by Hart *et al*. (2015) found that birds avoided temporal overlap with cicadas, suggesting that biotic noise (from sources including cicadas) may influence the frequency and timing of avian vocal signals. Many of the female elements and songs recorded and used for this experiment are produced at ≥3 KHz. Since these sounds fall within the range of high frequency noise, female sounds are at risk of being masked by cicada advertising calls, and background noise differences between

sites may explain why we observed a higher signal-to-noise ratio for female sounds played at the dry forest site (Slabbekoorn, 2004).

Source population

Source population had little influence on the degradation of male or female Rufous-and-white Wren elements. Only male elements showed a significant effect of source population for signal-to-noise ratio, where male elements recorded at the montane forest and wet forest sites showed less degradation than elements recorded at our dry forest site (i.e. higher signal-to-noise ratios), but for no other degradation measurements. Many animals increase signal-to-noise ratio to compensate for noisy environments (Brumm and Slabbekoorn, 2005). For instance abiotic features such as wind and fast-flowing rivers produce low-frequency noise (0.1-1.0 KHz for wind noise, up to 4 kHz for aquatic noise, Slabbekoorn 2004; Bradbury and Vehrencamp, 2011) that can mask signals in this range. Background noise differences among sites likely contributed to the higher signal-to-noise ratio observed for male elements from the wet and montane forest sites. For example, species living next to water produce vocalizations at higher frequencies so that they are not masked by the noise produced by streams (Martens and Geduldig, 1990). By comparison, there is less low-frequency ambient noise at the dry forest site during the breeding season, where there is little or no moving water, and conditions are less windy. The reduced background noise may explain why broadband elements are commonly used in songs at the dry forest site where males often terminate songs using broadband elements (e. g. the second male terminal element in the second row of Figure 2.1; 17 of 40 of song types recorded in 2012-13 included broadband terminal elements). By comparison, male elements (especially terminal elements, e.g. the fourth and fifth male terminal elements in the second row of Figure 2.1) from our wet and montane forest sites tend to be more tonal (Figure 2.1; only 2 of 35 song types at our wet forest site, while only 8 of 33 song types at our montane forest site included broadband terminal elements), suggesting that males use these

elements over broadband signals because they are masked less easily by ambient noise. Differences in signal-to-noise ratio of elements for male Rufous-and-white Wrens could be indicative of local adaptation, but could also represent phenotypic plasticity. For instance Red-wing Blackbirds (*Agelaius phoenicius*) make short-term modifications to their songs by increasing their signal tonality when exposed to low frequency white noise (Hanna *et al.,* 2011). Evidence from this study and others (Slabbekoorn and Peet, 2003; Mockford *et al.,* 2011; Parris and McCarthy, 2013; Gough *et al.,* 2014) have demonstrated the high plasticity in birds that learn their songs, where individuals are able to modify their songs in the presence of increased noise to stand out in their environment.

Female elements did not show differences in signal-to-noise ratio, in contrast to the pattern observed for males. However, we did observe significant differences for the other three degradation measurements; these results may indicate local adaptations for female elements. Tail-to-signal ratio was significant in our overall model, but did not show any differences among populations. Female elements from the montane forest site had a lower blur ratio overall than elements recorded from the other two sites. This is likely due to the fact that vegetation density is higher at the montane forest site, suggesting that female elements from this population are adapted for transmission through dense vegetation. Finally, we found small differences for the excess attenuation of female elements and songs, with sounds recorded from the wet forest site showing less excess attenuation than sounds recorded from the montane and dry forest sites. These differences may be indicate local adaptation, given that excess attenuation was highest at the wet forest site (although not significantly different than excess attenuation at our dry forest site). This result aligns with predictions of the Acoustic Adaptation Hypothesis, given that we would expect sounds from each of the three sites to be optimized to their respective sites.

Element type

Both male and female elements showed similar degradation patterns, suggesting that song elements have evolved under similar influences for both sexes. Nevertheless, degradation was not equal across all element types. For example, trill elements exhibited a lower signal-to-noise ratio (possibly because they are produced at lower frequencies than other elements and therefore more likely to be masked by background noise) than introductory and terminal elements, but experienced less blurring. It would be reasonable to predict that trill elements would experience less blurring, given that trill elements have lower frequencies and are more tonal than introductory or terminal elements (Figure 2.1) and therefore should experience less degradation (Brown and Handford, 2000; Slabbekoorn *et al.,* 2002). Our results for the interaction between element and distance supported this prediction; we observed little variation in the blurring of trill elements as transmission distance increased for both sexes. In contrast, terminal elements showed a higher blur ratio than did trill elements, and blur ratio increased with distance for terminal elements. However, both males and females appeared to compensate for this by singing terminal elements that had a higher signal-to-noise ratio (Table S2). By comparison, introductory elements fell in between terminal and trill elements with regards to signal-to-noise ratio and blur ratio, but male introductory elements experienced greater excess attenuation, while female introductory elements showed a greater tail-to-signal ratio; this suggests that trills are likely more important for long-distance communication (given that they experience less degradation over further distances, Barker *et al.,* 2009), whereas introductory elements and terminal elements are likely most important over shorter distances and potentially used by receivers to locate individuals at closer ranges (Morton, 1986). Additionally, these elements may aid receivers in determining the signaler's identity (Bee et al., 2001; Sandoval *et al.,* 2014), given that these components of the song are highly variable (unpublished data).

Male vs. female transmission

Sex of the signaler may play a role in the attenuation and degradation of animal signals, but to date the Acoustic Adaptation Hypothesis has primarily been tested only on male acoustic signals (Morton, 1975; Boncoraglio and Saino, 2007). The differences we found between the sexes in the degradation of song elements (i.e. source population significantly affected the signal-to-noise ratio of males versus blur ration and excess attenuation for females) may reflect differences in communication strategies between sexes (Langmore, 1998). Given that females tend to be less conspicuous than males when singing (females produce fewer songs, and sing primarily from lower perches in the understory; Topp and Mennill, 2008; Barker and Mennill, 2009), and that female songs degrade faster as distance increases (Barker *et al.,* 2009), this suggests that male songs and singing behaviour are likely better adapted for transmitting longer distances than females (Barker *et al.,* 2009; Barker and Mennill, 2009). Further, duetting is an important aspect of the vocal behaviour in this species (Mennill and Vehrencamp, 2005), and while the average distance between pairs when performing duets is approximately 20 m, the majority of duets are produced between 0 and 10 m (Mennill and Vehrencamp, 2008). These observations suggest that female signals are not adapted to maximize transmission distance but rather optimized to communicate through dense vegetation over shorter distances with their breeding partners, especially since female song is known to play a role in coordinating breeding activities (Ritchison, 1983; Sonnenschein and Rayer, 1983). At all three sites we have observed females producing songs and calls at the nest during nest building, and from the nest while incubating eggs or brooding young (Kovach, 2013). Given that females vocalize so much near or at the nest, they risk drawing the attention of potential predators from afar. Therefore female signals may be quieter and experience greater degradation with increasing distance because broadcasting loud far-reaching signals could be detrimental to their fitness. The Acoustic Adaptation Hypothesis often assumes that animal vocalizations are adapted to maximize transmission range

while minimizing degradation (Boncoraglio and Saino, 2007); however differences in the transmission properties of males and females (Barker *et al.,* 2009) may reflect different life history traits.

Past studies have emphasized the role that culture has on the evolution of songs through forces that include selection, learning biases, and drift (Lynch, 1996; Podos and Warren, 2007). Importantly, song transmission properties may affect learning, especially in light of a recent study by Peters *et al*. (2012) that suggested young birds preferentially learn the least-degraded songs. As mentioned previously, terminal elements at both our wet forest and montane forest sites tend to be more tonal (e.g. the third through fifth male terminal elements in the second row of Figure 2.1) than at our dry forest site, where birds use terminal elements with sharp rising or falling frequency sweeps (e.g. the second male terminal element in the second row of Figure 2.1). Differences in the transmission properties of different element types could explain element differences among our three sites; ongoing research will explore differences in elements among these and other sites (Graham and Mennill, unpublished data).

Conclusion

Our study does not suggest that acoustic variation among the three populations of Rufous-and-white Wrens has been driven heavily by acoustic adaptation to three different tropical forest environments. While previous research makes it clear that these birds' songs are adapted for transmission through forests versus fields (Barker et al., 2008), our current work does not suggest that they are specifically adapted to different types of forest. We found that playback site (in particular ambient noise) played an important role in the transmission and degradation of both male and female elements. In contrast, source population had a weak effect on the degradation of elements for both males and females. Furthermore the interaction between playback site and source population did not suggest local adaptation, given that song elements did not transmit better

at their respective sites. While male and female elements showed similar patterns of degradation, we did observe a few important differences. For example male elements appeared to be optimized to transmit most efficiently through their environment, given that we found elements recorded from populations where ambient noise is higher had a higher signal-to-noise ratio. In contrast female elements showed no differences in signal-to-noise ratio among sites. However we did observe that source population affected blur ratio and excess attenuation of female elements. Elements recorded from the montane forest site (the habitat with the highest vegetation density) had a lower blur ratio, suggesting that these elements are optimized for transmission through densely vegetated habitat. While our observations of male and female elements do not suggest local adaptations, they may indicate of plastic modifications, but further studies are necessary to support this idea. Importantly this study emphasizes the transmission differences between sexes, which likely reflects behavioural and life history differences between sexes. Whereas male song elements are likely maximized for long-range transmission, this does not seem to be the case for female songs; female song elements seem to be optimized for transmission through dense vegetation. This is important given that females often sing from the densely vegetated understory and will also sing songs when they are concealed in their nests. Future studies should continue to compare male and female songs and singing strategies to not only increase our understanding of the function of female song (Reibel *et al.* 2003) but to better understand the behaviour and ecology of birds overall.

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Tables

Table 2.1: Main effects and two-factor interactions for linear mixed models analyzing male song elements for each of four measures of degradation of Rufous-and-white Wren song elements. The significance of bold values is ($p<0.05$).

Male Elements	Signal-to-noise ratio			Tail-to-signal ratio			Blur-ratio			Excess attenuation		
	df	**F**	**p**	**df**	**F**	**P**	**df**	**F**	**p**	**df**	**F**	**p**
Model	39	109.18	**<0.001**	39	23.61	**<0.001**	39	7.1	**<0.001**	39	75.02	**<0.001**
Playback site	2	61.03	**<0.001**	2	15.78	**<0.001**	2	0.12	0.889	2	81.17	**<0.001**
Source population	2	11.33	**<0.001**	2	1.33	0.266	2	1.99	0.137	2	0.03	0.974
Distance	3	967.13	**<0.001**	3	222.03	**<0.001**	3	24.05	**<0.001**	3	749.43	**<0.001**
Element type	2	8.70	**<0.001**	2	2.77	0.063	2	3.58	**0.028**	2	13.48	**<0.001**
Playback site*Source population	4	3.67	**<0.001**	4	0.17	0.951	4	0.53	0.715	4	2.30	0.057
Playback site*Distance	6	36.88	**<0.001**	6	4.72	**<0.001**	6	2.36	**0.029**	6	37.36	**<0.001**
Playback site*Element type	4	12.74	**<0.001**	4	1.65	0.158	4	2.29	0.058	4	2.37	0.051
Source population*Distance	6	1.53	0.165	6	0.99	0.432	6	0.68	0.665	6	1.26	0.275
Source population*Element type	4	21.35	**<0.001**	4	11.45	**<0.001**	4	4.85	**0.001**	4	4.67	**0.001**
Distance*Element type	6	2.27	**0.034**	6	0.46	0.838	6	3.22	**0.004**	6	2.01	0.062

Table 2.2: Main effects and two-factor interactions for linear mixed models analyzing female song elements for each of four measures of degradation of Rufous-and-white Wren song elements. The significance of bold values is ($p<0.05$).

Female Elements	Signal-to-noise ratio			Tail-to-signal ratio			Blur-ratio			Excess attenuation		
	df	F	p	df	F	P	df	F	p	df	F	p
Model	39	126.55	**<0.001**	39	80.45	**<0.001**	39	32.29	**<0.001**	39	58.93	**<0.001**
Playback site	2	84.78	**<0.001**	2	27.36	**<0.001**	2	8.48	**<0.001**	2	50.30	**<0.001**
Source population	2	0.27	0.763	2	3.09	**0.046**	2	9.59	**<0.001**	2	7.83	**<0.001**
Distance	3	1073.53	**<0.001**	3	527.32	**<0.001**	3	88.86	**<0.001**	3	574.27	**<0.001**
Element type	2	25.99	**<0.001**	2	59.99	**<0.001**	2	24.71	**<0.001**	2	0.99	0.373
Playback site*Source population	4	5.78	**<0.001**	4	1.69	0.151	4	1.74	0.140	4	0.60	0.660
Playback site*Distance	6	28.53	**<0.001**	6	9.20	**<0.001**	6	8.17	**<0.001**	6	12.67	**<0.001**
Playback site*Element type	4	15.12	**<0.001**	4	0.93	0.448	4	1.57	0.179	4	1.10	0.356
Source population*Distance	6	0.51	0.804	6	1.09	0.368	6	5.57	**<0.001**	6	1.26	0.274
Source population*Element type	4	33.29	**<0.001**	4	242.51	**<0.001**	4	68.90	**<0.001**	4	1.06	0.374
Distance*Element type	6	1.54	0.162	6	2.33	**0.030**	6	4.21	**<0.001**	6	1.11	0.355

Table 2.3: Main effects and two-factor interactions for the linear mixed models analyzing comparisons of background noise during male and female song elements. The significance of bold values is ($p<0.05$).

	Male song elements			Female song elements		
	df	**F**	**p**	**df**	**F**	**p**
Model	39	26.02	**<0.001**	12	32.92	**<0.001**
Playback site	2	6.99	**0.001**	2	7.47	**0.001**
Source population	2	0.11	0.899	2	0.20	0.822
Distance	3	155.39	**<0.001**	3	189.64	**<0.001**
Element type	2	1.71	0.182	2	1.87	0.155

Figures

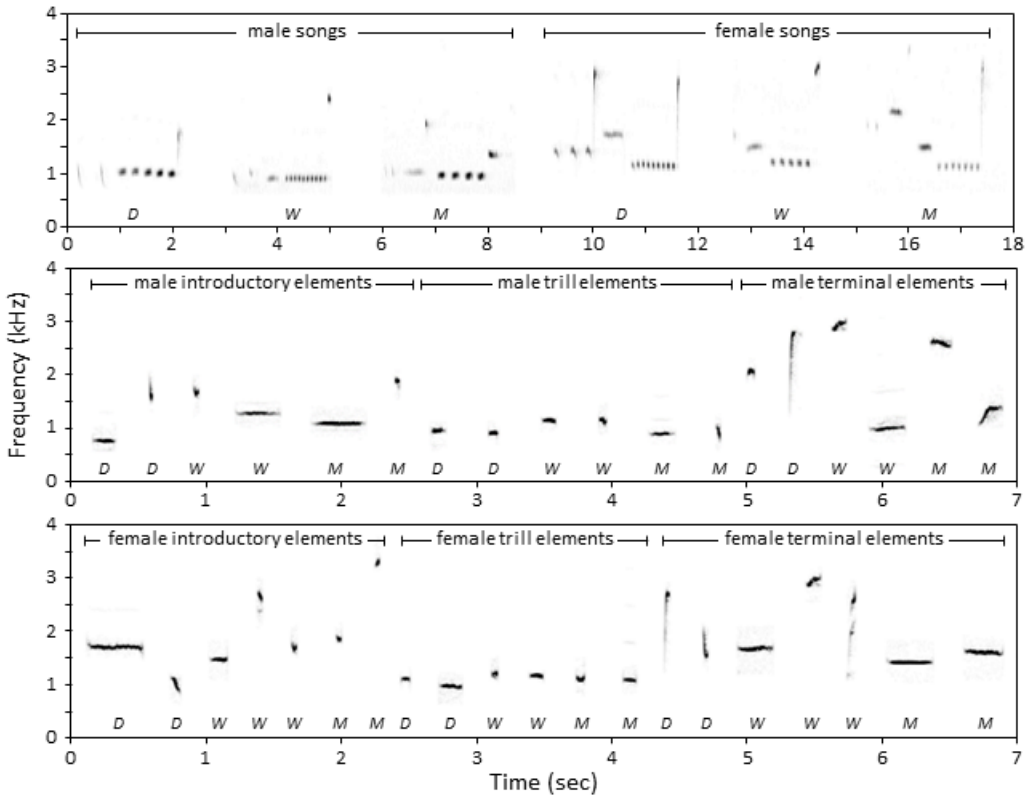

Figure 2.1: Sound spectrograms of example male and female Rufous-and-White Wren songs recorded from each of the three populations where playback experiments were conducted (top row). Sound spectrograms of example male song elements (second row) and female song elements (third row) used for playback during the transmission experiment. Letters indicate the population where the song or song element was recorded (D = Dry Forest, W = Wet Forest, and M = Montane Forest).

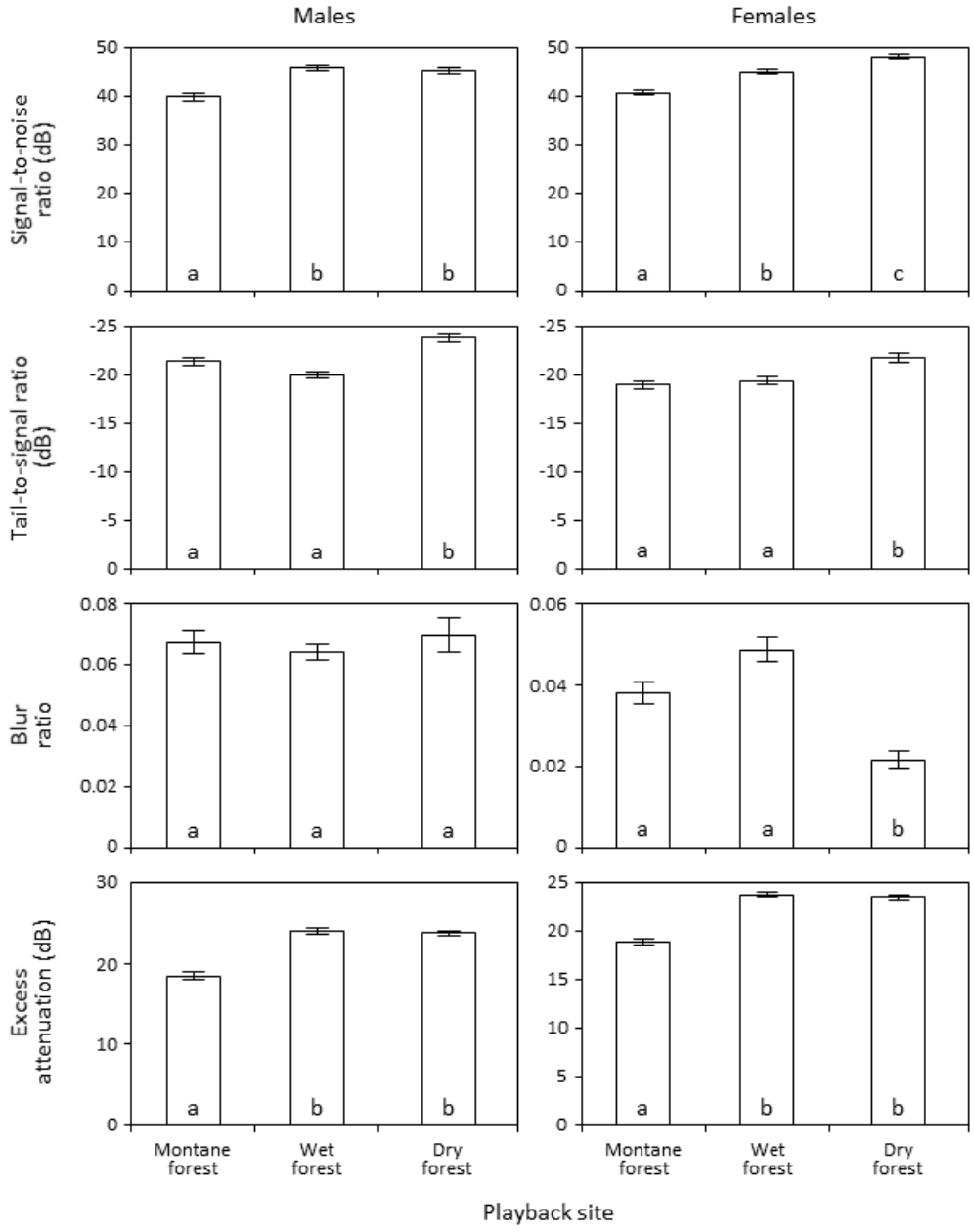

Figure 2.2: Four measurements of sound degradation of Rufous-and-white Wren song elements at each of three different playback sites in Costa Rica, both for males (left column) and females (right column). Error bars are standard errors of the mean, and bars with different letters indicate that values are significantly different from each other in post-hoc tests.

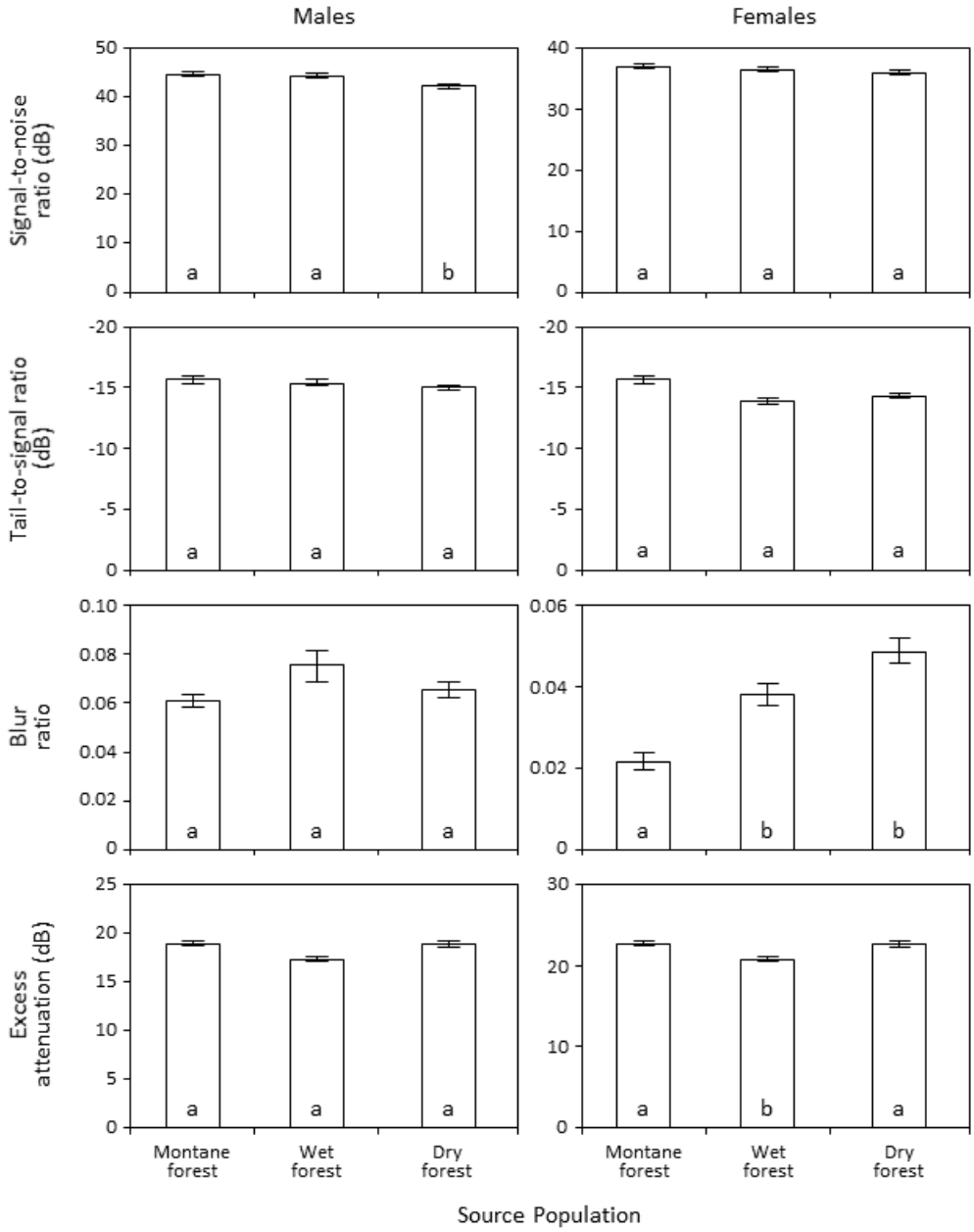

Figure 2.3: Four measurements of sound degradation of Rufous-and-white Wren song elements based on the source population (where a sound was recorded), both for males (left column) and females (right column). Error bars are standard errors of the mean, and bars with different letters indicate that values are significantly different from each other in post-hoc tests.

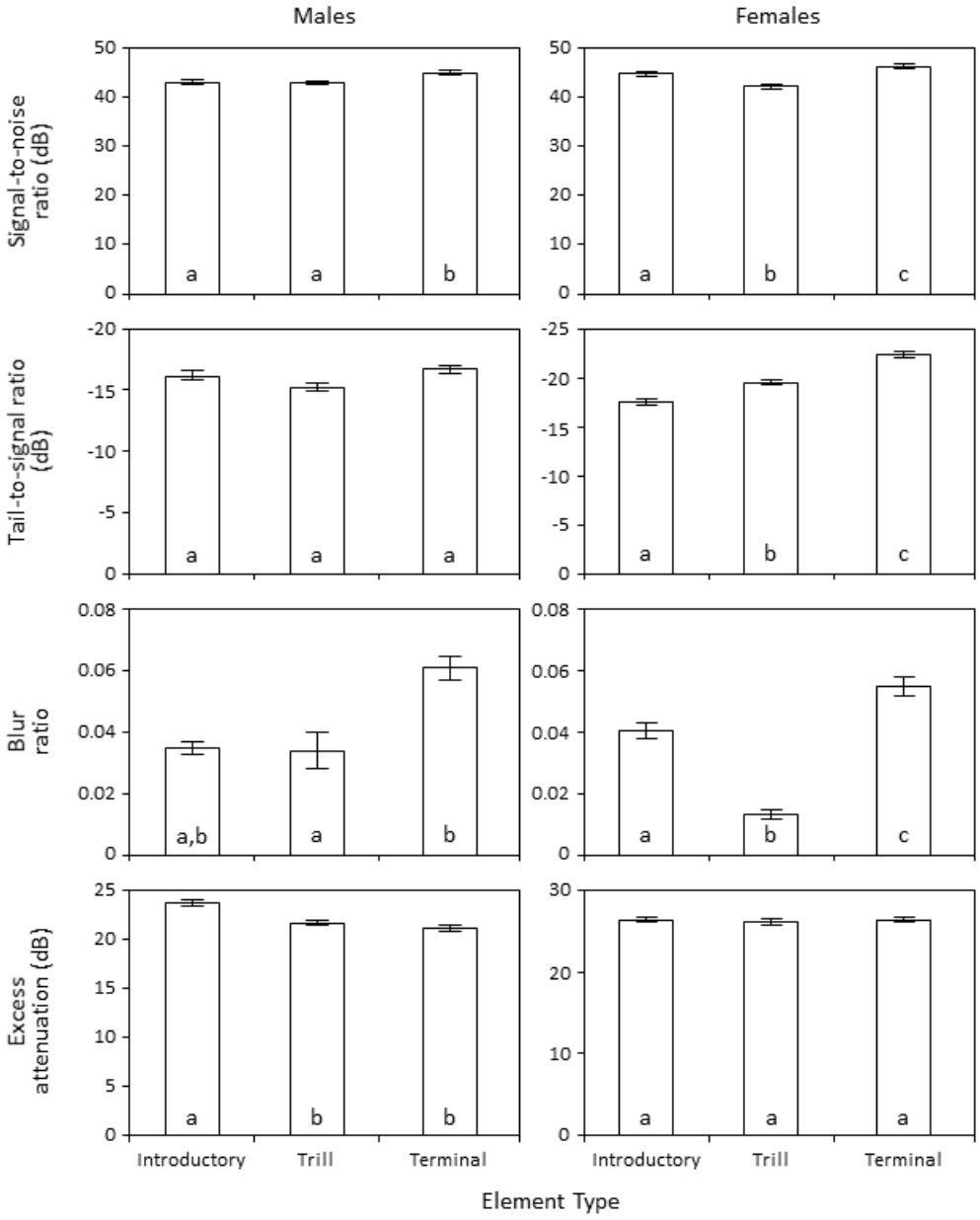

Figure 2.4: Four measurements of sound degradation of Rufous-and-white Wren song elements based on element type for males (left column) and females (right column). Error bars are standard errors of the mean, and bars with different letters indicate that values are significantly different from each other in post-hoc tests.

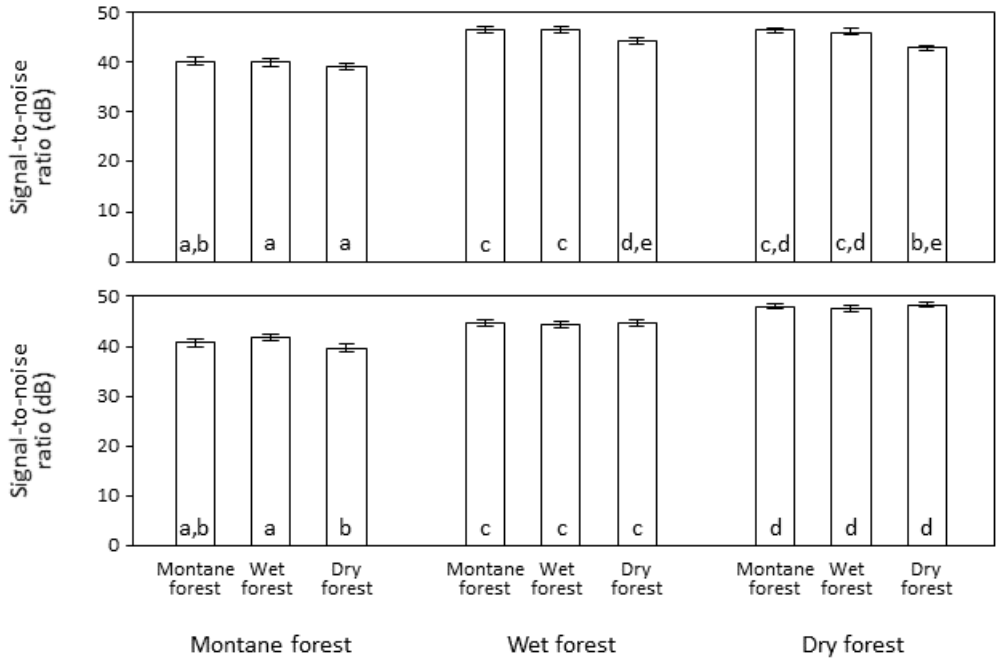

Figure 2.5: Signal-to-noise ratio measurements of Rufous-and-white Wren song elements showing the interaction of playback site × source population for males (top) and females (bottom). Error bars are standard errors of the mean, and bars with different letters indicate that values are significantly different from each other in post-hoc tests.

Supplementary information accompanying Chapter 2

Supplementary Methods: Filters used in Adobe Audition (version 3.0) to isolate male and female sounds.

Male sounds: introductory element 1: 0.5-1.0 kHz; introductory element 2: 1.2-2.2 kHz; introductory element 3: 1.2-2.0 kHz; introductory element 4: 1.0-1.5 kHz; introductory element 5: 0.5-1.4 kHz; introductory element 6: 1.5-2.1 kHz; trill element 1: 0.5-1.1 kHz; trill element 2: 0.5-1.2 kHz; trill element 3: 0.9-1.2 kHz; trill element 4: 0.9-1.5 kHz; trill element 5: 0.5-1.2 kHz; trill element 6: 0.5-1.3; terminal element 1: 1.8-2.6 kHz; terminal element 2: 1.0-3.0 kHz; terminal element 3: 2.8-3.2 kHz; terminal element 4: 0.5-1.5 kHz; terminal element 5: 2.1-2.8 kHz; terminal element 6: 1.0-1.8 kHz; Dry Forest song 1: 0.5-2.4 kHz; Dry Forest song 2: 0.5-3.0 kHz; Wet Forest song 1: 0.5-2.6 kHz; Wet Forest song 2: 0.5-2.1 kHz; Montane Forest song 1: 0.5-2.9 kHz; Montane Forest song 2: 0.5-2.8 kHz.

Female sounds: introductory element 7: 1.1-2.1 kHz; introductory element 8: 0.9-1.2 kHz; introductory element 9: 1.0-1.8 kHz; introductory element 10: 2.0-3.0 kHz; introductory element 11: 1.5-2.0 kHz; introductory element 12: 1.8-2.0 kHz; introductory element 13: 3.0-3.8 kHz; trill element 7: 0.9-1.2 kHz; trill element 8: 0.9-1.2 kHz; trill element 9: 1.0-1.5 kHz; trill element 10: 1.0-1.2 kHz; trill element 11: 0.5-1.8 kHz; trill element 12: 0.8-1.3 kHz; terminal element 7: 1.2-3.0 kHz; terminal element 8: 1.2-2.0 kHz; terminal element 9: 1.2-2.0 kHz; terminal element 10: 2.5-3.2 kHz; terminal element 11: 1.0-3.0 kHz; terminal element 12: 1.0-1.8 kHz; terminal element 13: 1.0-1.9 kHz; Dry Forest song 3: 0.9-3.2 kHz; Dry Forest song 4: 0.7-2.2 kHz; Wet Forest song 3: 0.8-3.0 kHz; Wet Forest song 4: 0.9-3.2 kHz; Montane Forest song 3: 0.8-3.8 kHz; Montane Forest song 4: 0.7-2.2 kHz.

Table 2.S1: Results for the four measurements of degradation of male song elements for remaining main effects and two-way interactions not presented in the main body of the text. Values presented are mean ± standard error. Results of post-hoc test are presented in brackets; within each separate section of the table, values are significantly different from each other if they exhibit a different letter.

Male Elements	**Signal-to-Noise Ratio**		**Tail-to-Signal Ratio**		**Blur Ratio**		**Excess Attenuation**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Distance								
5	43.65 (a)	0.27	-21.63 (a)	0.34	0.043 (a)	0.005	22.13 (a)	0.23
10	39.09 (b)	0.26	-17.28 (b)	0.29	0.053 (a)	0.002	20.26 (b)	0.27
20	30.22 (c)	0.35	-12.85 (c)	0.30	0.087 (b)	0.004	32.43 (c)	0.27
40	27.22 (d)	0.27	-10.70 (d)	0.32	0.090 (b)	0.009	30.90 (d)	0.30
Playback Site x Distance								
5								
Montane Forest	39.90 (b)	0.54	-21.24 (h)	0.58	0.047 (d,e)	0.004	17.99 (f)	0.50
Wet Forest	45.84 (a)	0.31	-19.92 (f,g)	0.44	0.043 (d,e)	0.003	24.26 (e)	0.28
Dry Forest	45.21 (a)	0.44	-23.66 (h)	0.68	0.041 (e)	0.012	24.15 (e)	0.22
10								
Montane Forest	36.08 (c)	0.58	-17.98 (e,f)	0.73	0.056 (c,d,e)	0.004	14.80 (g)	0.72
Wet Forest	40.39 (b)	0.32	-16.69 (d,e)	0.36	0.05 (d,e)	0.004	23.08 (e)	0.19
Dry Forest	40.80 (b)	0.38	-17.45 (e)	0.48	0.053 (d,e)	0.004	22.96 (e)	0.17
20								
Montane Forest	23.55 (g)	0.50	-12.37 (b)	0.55	0.096 (a,b)	0.009	32.43 (b)	0.40
Wet Forest	31.34 (d)	0.39	-11.36 (b)	0.37	0.089 (a,b,c)	0.006	34.48 (a)	0.61
Dry Forest	35.75 (c)	0.40	-14.65 (c,d)	0.56	0.077 (b,c,d)	0.006	30.47 (c)	0.28
40								
Montane Forest	27.51 (e,f)	0.61	-13.46 (b,c)	0.69	0.067 (a,b,c,d,e)	0.007	27.17 (d)	0.44
Wet Forest	25.62 (f)	0.43	-8.42 (a)	0.36	0.094 (a,b,c)	0.008	32.66 (a,b)	0.53
Dry Forest	28.23 (f)	0.40	-11.34 (b)	0.50	0.117 (a)	0.018	32.85 (a,b)	0.39
Playback Site x Element Type								
Introductory Elements								
Montane Forest	38.39 (c)	0.77	-15.64 (a)	0.78	0.066 (a)	0.005	24.8 (a)	0.84
Wet Forest	45.52 (a,b)	0.64	-15.13 (a)	0.49	0.048 (a)	0.003	29.78 (a)	0.55
Dry Forest	45.15 (a,b)	0.63	-17.47 (a)	0.66	0.049 (a)	0.004	28.49 (a)	0.38
Trill Elements								
Montane Forest	38.28 (c)	0.63	-15.92 (a)	0.56	0.032 (a)	0.003	22.43 (a)	0.70
Wet Forest	45.64 (a,b)	0.59	-13.71 (a)	0.40	0.044 (a)	0.004	27.77 (a)	0.49
Dry Forest	44.94 (a,b)	0.49	-16.13 (a)	0.57	0.06 (a)	0.015	27.17 (a)	0.34

Terminal Elements								
Montane Forest	43.03 (b)	0.85	-17.45 (a)	0.53	0.107 (a)	0.009	22.79 (a)	0.85
Wet Forest	46.36 (a)	0.68	-15.47 (a)	0.50	0.104 (a)	0.006	26.51 (a)	0.47
Dry Forest	45.53 (a,b)	0.53	-17.37 (a)	0.49	0.102 (a)	0.005	26.54 (a)	0.37
Source Population x Distance								
5								
Montane Forest	45.07 (a)	0.37	-21.76 (a)	0.79	0.034 (a)	0.002	22.69 (a)	0.40
Wet Forest	44.76 (a)	0.42	-22.17 (a)	0.44	0.056 (a)	0.014	22.52 (a)	0.34
Dry Forest	42.41 (a)	0.55	-20.99 (a)	0.50	0.039 (a)	0.003	22.65 (a)	0.43
10								
Montane Forest	40.09 (a)	0.36	-17.85 (a)	0.40	0.053 (a)	0.004	21.25 (a)	0.45
Wet Forest	40.22 (a)	0.39	-18.01 (a)	0.53	0.054 (a)	0.004	20.25 (a)	0.48
Dry Forest	38.02 (a)	0.53	-16 (a)	0.55	0.05 (a)	0.004	21.27 (a)	0.47
20								
Montane Forest	30.67 (a)	0.54	-13.93 (a)	0.66	0.079 (a)	0.005	32.68 (a)	0.45
Wet Forest	31.34 (a)	0.59	-12.7 (a)	0.47	0.087 (a)	0.008	31.79 (a)	0.46
Dry Forest	29.15 (a)	0.66	-11.91 (a)	0.36	0.085 (a)	0.008	32.71 (a)	0.48
40								
Montane Forest	28.78 (a)	0.33	-12.05 (a)	0.59	0.084 (a)	0.006	31.01 (a)	0.47
Wet Forest	27.07 (a)	0.42	-10.8 (a)	0.50	0.114 (a)	0.025	31.85 (a)	0.55
Dry Forest	25.79 (a)	0.58	-9.24 (a)	0.52	0.098 (a)	0.009	32.47 (a)	0.52
Source Population x Element Type								
Introductory Elements								
Montane Forest	36.38 (a)	0.60	-21.91 (a,b)	0.75	0.022 (a,b)	0.004	24.24 (a)	0.57
Wet Forest	38.08 (a)	0.65	-22.01 (a,b)	0.61	0.041 (a,b)	0.004	24.02 (a,b)	0.54
Dry Forest	33.63 (a)	0.81	-20.24 (a)	0.55	0.043 (a,b)	0.004	22.72 (a,b,c)	0.64
Trill Elements								
Montane Forest	35.17 (a)	0.60	-20.41 (a)	0.57	0.032 (a,b)	0.003	21.43 (c)	0.48
Wet Forest	34.83 (a)	0.64	-20.10 (a)	0.38	0.061 (a)	0.017	21.41 (c)	0.55
Dry Forest	35.28 (a)	0.60	-22.16 (a,b)	0.59	0.001 (b)	0.004	22.13 (a,b,c)	0.52
Terminal Elements								
Montane Forest	38.79 (a)	0.63	-22.89 (a,b)	0.50	0.050 (a,b)	0.004	20.90 (c)	0.55
Wet Forest	36.58 (a)	0.67	-24.22 (b)	0.56	0.067 (a)	0.007	20.78 (c)	0.56
Dry Forest	34.84 (a)	0.69	-20.51 (a)	0.43	0.066 (a)	0.007	21.56 (b,c)	0.55

Element Type x Distance								
Introductory Elements								
5	43.46 (b)	0.59	-21.48 (a)	0.67	0.035 (e,f)	0.003	24.05 (a)	0.47
10	40.13 (c)	0.53	-17.56 (a)	0.58	0.038 (d,e,f)	0.002	21.86 (a)	0.42
20	30.30 (d)	0.66	-12.90 (a)	0.70	0.072 (c,d)	0.005	34.39 (a)	0.48
40	27.79 (e)	0.57	-11.24 (a)	0.60	0.071 (c,d,e)	0.006	32.76 (a)	0.54
Trill Elements								
5	43.54 (b)	0.31	-20.89 (a)	0.60	0.034 (e,f)	0.013	22.24 (a)	0.35
10	38.72 (c)	0.36	-16.69 (a)	0.43	0.027 (f)	0.002	20.63 (a)	0.43
20	30.03 (d)	0.55	-12.52 (a)	0.37	0.048 (d,e,f)	0.005	31.03 (a)	0.41
40	26.40 (e)	0.36	-9.61 (a)	0.58	0.074 (c,d,e)	0.023	31.52 (a)	0.55
Terminal Elements								
5	45.22 (a)	0.44	-22.53 (a)	0.49	0.061 (c,d,e,f)	0.004	21.54 (a)	0.30
10	39.45 (c)	0.41	-17.59 (a)	0.49	0.093 (b,c)	0.005	20.27 (a)	0.53
20	30.92 (d)	0.58	-13.18 (a)	0.37	0.141 (a)	0.010	31.66 (a)	0.44
40	27.58 (e)	0.48	-11.44 (a)	0.41	0.133 (a,b)	0.008	31.02 (a)	0.41

Table 2.S2: Results for the four measurements of degradation of female song elements for remaining main effects and two-way interactions not presented in the main body of the text. Values presented are mean ± standard error. Results of post-hoc test are presented in brackets; within each separate section of the table, values are significantly different from each other are exhibit a different letter.

Female Elements	**Signal-to-Noise Ratio**		**Tail-to-Signal Ratio**		**Blur Ratio**		**Excess Attenuation**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Distance								
5	44.54 (a)	0.25	-19.79 (a)	0.25	0.036 (a)	0.002	22.07 (a)	0.20
10	39.22 (b)	0.30	-16.04 (b)	0.24	0.055 (b)	0.003	20.25 (b)	0.25
20	30.52 (c)	0.35	-10.85 (c)	0.25	0.089 (c)	0.004	31.58 (c)	0.34
40	27.16 (d)	0.28	-10.06 (d)	0.28	0.088 (d)	0.004	31.60 (c)	0.28
Playback Site x Distance								
5								
Montane Forest	40.81 (c)	0.43	-18.63 (e)	0.51	0.039 (d,e)	0.004	18.44 (d)	0.43
Wet Forest	44.69 (b)	0.34	-19.06 (e)	0.38	0.046 (d)	0.004	23.96 (c)	0.29
Dry Forest	48.12 (a)	0.37	-21.67 (f)	0.40	0.023 (e)	0.002	23.82 (c)	0.21
10								
Montane Forest	37.18 (d)	0.50	-16.68 (d)	0.48	0.055 (c,d)	0.004	14.99 (e)	0.64
Wet Forest	37.99 (d)	0.48	-15.23 (d)	0.40	0.053 (c,d)	0.004	23.24 (c)	0.19
Dry Forest	42.49 (c)	0.46	-16.15 (d)	0.38	0.056 (c,d)	0.004	22.53 (c)	0.22
20								
Montane Forest	23.77 (f)	0.44	-10.59 (b,c)	0.46	0.102 (a)	0.007	29.14 (b)	0.61
Wet Forest	30.70 (e)	0.41	-9.98 (c)	0.42	0.088 (a,b)	0.006	35.06 (a)	0.57
Dry Forest	37.10 (d)	0.41	-11.97 (c)	0.40	0.070 (b,c)	0.006	30.58 (a)	0.46
40								
Montane Forest	25.96 (f)	0.46	-11.92 (b,c)	0.61	0.063 (b,c,d)	0.008	28.21 (b)	0.53
Wet Forest	25.39 (f)	0.50	-8.31 (a)	0.48	0.093 (a)	0.007	33.27 (a)	0.47
Dry Forest	30.13	0.34	-9.95 (a,b)	0.38	0.092 (a)	0.006	33.25 (a)	0.39
Playback Site x Element Type								
Introductory Elements								
Montane Forest	42.02 (d)	0.72	-12.66 (a)	0.46	0.078 (a)	0.01	22.73 (a)	0.74
Wet Forest	44.34 (b,c)	0.58	-12.59 (a)	0.41	0.069 (a)	0.00	28.22 (a)	0.52
Dry Forest	48.38 (a)	0.55	-13.45 (a)	0.42	0.067 (a)	0.00	27.25 (a)	0.50
Trill Elements								
Montane Forest	37.08 (e)	0.66	-14.51 (a)	0.46	0.032 (a)	0.00	21.69 (a)	0.71
Wet Forest	44.21 (b,c,d)	0.62	-13.37 (a)	0.42	0.042 (a)	0.00	27.81 (a)	0.54
Dry Forest	45.93 (b,c)	0.49	-14.82 (a)	0.37	0.026 (a)	0.00	27.44 (a)	0.31

Terminal Elements								
Montane Forest	44.34 (c,d)	0.71	-16.34 (a)	0.58	0.098 (a)	0.01	22.85 (a)	0.73
Wet Forest	45.52 (b)	0.62	-15.65 (a)	0.50	0.091 (a)	0.01	28.24 (a)	0.45
Dry Forest	50.06 (a)	0.58	-17.22 (a)	0.50	0.089 (a)	0.01	27.10 (a)	0.39
Source Population x Distance								
5								
Montane Forest	45.09 (a)	0.49	-20.67 (a)	0.60	0.022 (d)	0.003	23.24 (a)	0.37
Wet Forest	45.29 (a)	0.36	-19.79 (a)	0.33	0.038 (c,d)	0.003	21.34 (a)	0.31
Dry Forest	44.91 (a)	0.48	-19.43 (a)	0.37	0.049 (b,c)	0.003	23.11 (a)	0.37
10								
Montane Forest	40.07 (a)	0.55	-17.04 (a)	0.49	0.035 (c,d)	0.003	21.23 (a)	0.45
Wet Forest	39.29 (a)	0.46	-15.08 (a)	0.38	0.065 (c,d)	0.004	20.19 (a)	0.41
Dry Forest	39.32 (a)	0.55	-15.86 (a)	0.37	0.064 (c,d)	0.005	21.30 (a)	0.43
20								
Montane Forest	31.53 (a)	0.65	-12.23 (a)	0.56	0.067 (b)	0.005	32.05 (a)	0.62
Wet Forest	30.64 (a)	0.52	-10.05 (a)	0.36	0.095 (a,b)	0.006	30.98 (a)	0.48
Dry Forest	30.45 (a)	0.64	-11.08 (a)	0.38	0.098 (a)	0.007	31.75 (a)	0.66
40								
Montane Forest	28.5 (a)	0.53	-11.08 (a)	0.64	0.039 (c,d)	0.004	32.01 (a)	0.51
Wet Forest	27.73 (a)	0.42	-9.06 (a)	0.33	0.097 (a)	0.007	31.48 (a)	0.41
Dry Forest	27.45 (a)	0.50	-10.14 (a)	0.49	0.112 (a)	0.008	33.51 (a)	0.56
Source Population x Element Type								
Introductory Elements								
Montane Forest	42.36 (d)	0.71	-14.07 (a)	0.38	0.039 (c)	0.005	26.76 (a)	0.66
Wet Forest	46.17 (a,b)	0.55	-17.51 (a)	0.37	0.034 (c)	0.004	25.6 (a)	0.49
Dry Forest	46.20 (a,b)	0.67	-20.96 (a)	0.41	0.048 (b,c)	0.004	27.29 (a)	0.66
Trill Elements								
Montane Forest	43.06 (c,d)	0.62	-18.96 (a)	0.42	0.026 (c,d)	0.003	26.39 (a)	0.52
Wet Forest	42.64 (d)	0.64	-19.36 (a)	0.41	0.006 (d,e)	0.003	24.94 (a)	0.53
Dry Forest	41.52 (d)	0.66	-20.26 (a)	0.41	0.005 (a)	0.003	26.93 (a)	0.55
Terminal Elements								
Montane Forest	48.34 (a)	0.69	-28.33 (a)	0.43	0.010 (c,d)	0.002	26.78 (a)	0.55
Wet Forest	45.33 (b,c)	0.58	-21.895 (a)	0.43	0.090 (a,b)	0.005	25.88 (a)	0.46
Dry Forest	45.25 (b,c)	0.74	-16.778 (a)	0.41	0.070 (a)	0.006	26.87 (a)	0.59
Distance x Element Type								
5								
Introductory Elements	45.47 (a)	0.40	-17.72 (a)	0.38	0.040 (f,g)	0.003	22.7 (a)	0.40
Trill Elements	43.07 (a)	0.39	-19.64 (a)	0.31	0.013 (h)	0.001	22.13 (a)	0.34

Terminal Elements	46.52 (a)	0.45	-22.42 (a)	0.48	0.055 (e,f)	0.004	22.43 (a)	0.31
10								
Introductory Elements	40.27 (a)	0.47	-14.41 (a)	0.38	0.060 (d,e)	0.004	20.54 (a)	0.42
Trill Elements	37.03 (a)	0.36	-15.76 (a)	0.30	0.028 (g,h)	0.002	21.03 (a)	0.45
Terminal Elements	40.95 (a)	0.60	-17.49 (a)	0.49	0.076 (c,d)	0.005	20.96 (a)	0.43
20								
Introductory	30.81 (a)	0.55	-9.21 (a)	0.39	0.097 (b)	0.006	31.96 (a)	0.68
Trill Elements	28.89 (a)	0.60	-11.06 (a)	0.35	0.046 (e,f,g)	0.004	30.58 (a)	0.55
Terminal Elements	32.59 (a)	0.60	-12.58 (a)	0.48	0.117 (a)	0.007	31.99 (a)	0.50
40								
Introductory Elements	28.18 (a)	0.41	-8.98 (a)	0.43	0.088 (b,c)	0.006	32.73 (a)	0.56
Trill Elements	25.46 (a)	0.38	-9.49 (a)	0.44	0.041 (e,f,g)	0.004	31.68 (a)	0.49
Terminal Elements	29.96 (a)	0.54	-11.55 (a)	0.54	0.120 (a)	0.009	32.34 (a)	0.41

Table 2.S3: Main effects and two-factor interactions for the linear mixed models analyzing the full songs of males (top) and females (bottom) for each measurement of degradation.

Male Full Songs	**Signal-to-Noise Ratio**			**Tail-Signal Ratio**			**Blur Ratio**			**Excess Attenuation**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Model	23	43.15	**<0.001**	23	14.63	**<0.001**	23	24.07	**<0.001**	23	43.29	**<0.001**
Playback Site	2	15.42	**<0.001**	2	0.25	0.782	2	3.87	**0.021**	2	37.6	**<0.001**
Source Population	2	0.49	0.614	2	2.78	0.063	2	0.71	0.492	2	0.95	0.386
Distance	3	245.7	**<0.001**	3	88.59	**<0.001**	3	103.76	**<0.001**	3	252.25	**<0.001**
Playback site*Source Population	4	0.77	0.545	4	2.05	0.086	4	5.22	**<0.001**	4	0.95	0.433
Playback site*Distance	6	6.97	**<0.001**	6	1.34	0.237	6	15.06	**<0.001**	6	6.87	**<0.001**
Source Population*Distance	6	0.56	0.764	6	0.55	0.77	6	4.31	**<0.001**	6	0.5	0.811
Female Full Songs												
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Model	23	40.54	**<0.001**	23	24.72	**<0.001**	23	17.43	**<0.001**	23	28.93	**<0.001**
Playback Site	2	19.81	**<0.001**	2	9.21	**<0.001**	2	4.17	**0.016**	2	18.81	**<0.001**
Source Population	2	2.69	0.069	2	0.84	0.433	2	3.05	0.048	2	3.51	**0.031**
Distance	3	192.15	**<0.001**	3	159.71	**<0.001**	3	70.25	**<0.001**	3	174.56	**<0.001**
Playback Site*Source Population	4	1.82	0.124	4	1.4	0.232	4	1.18	0.317	4	0.13	0.971
Playback Site*Distance	6	8.62	**<0.001**	6	2.52	**0.021**	6	3.33	**0.003**	6	2.67	**0.015**
Source Population*Distance	6	0.94	0.467	6	0.23	0.968	6	4.76	**<0.001**	6	0.16	0.987

Table 2.S4: Results for the four measurements of degradation of male full songs for all main effects and two-way interactions. Values presented are mean ± standard error. Results of post-hoc test are presented in brackets; within each separate section of the table, values are significantly different from each other are exhibit a different letter. Whereas the main text focuses on analyses of playback of isolated elements of wren songs, this analysis focuses on playback of entire songs.

Male Full Songs	**Signal-to-Noise Ratio**		**Tail-to-Signal Ratio**		**Blur Ratio**		**Excess Attenuation**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Playback Site								
Montane Forest	28.76 (b)	0.63	-23.59 (a)	0.74	0.050 (a,b)	0.006	16.97 (a)	0.66
Wet Forest	34.40 (a)	0.62	-22.28 (a)	0.44	0.072 (b)	0.006	23.52 (b)	0.51
Dry Forest	33.24 (a)	0.58	-23.87 (a)	0.48	0.044 (a)	0.006	23.19 (b)	0.29
Source Population								
Montane Forest	24.95 (a)	0.61	-22.06 (a)	0.40	0.126 (a)	0.006	25.07 (a)	0.49
Wet Forest	25.43 (a)	0.68	-24.80 (a)	0.60	0.088 (a)	0.004	25.24 (a)	0.51
Dry Forest	24.23 (a)	0.64	-22.81 (a)	0.57	0.114 (a)	0.007	25.61 (a)	0.53
Distance								
5	32.13 (a)	0.59	-28.81(a)	0.67	0.056 (a)	0.003	21.23 (a)	0.33
10	26.40 (b)	0.36	-23.85 (b)	0.30	0.093 (b)	0.004	20.19 (a)	0.44
20	19.39 (c)	0.51	-19.67 (c)	0.43	0.145 (c)	0.006	30.70 (b)	0.41
40	15.96 (d)	0.41	-18.37 (c)	0.41	0.174 (d)	0.012	29.58 (b)	0.52
Playback Site x Source Population								
Montane Forest								
Montane Forest	20.17 (a)	1.25	-23.18 (a)	0.80	0.071 (a,b,c)	0.013	21.83 (a)	1.21
Wet Forest	21.17 (a)	1.01	-23.73 (a)	1.94	0.044 (a,b,c)	0.005	21.05 (a)	1.13
Dry Forest	20.57 (a)	1.05	-23.88 (a)	0.69	0.035 (b,c)	0.009	21.14 (a)	1.12
Wet Forest								
Montane Forest	25.46 (a)	1.02	-20.43 (a)	0.67	0.061 (a,b,c)	0.008	26.93 (a)	0.81
Wet Forest	26.36 (a)	0.98	-24.29 (a)	0.62	0.074 (a,b)	0.009	27.66 (a)	0.89
Dry Forest	24.06 (a)	1.19	-22.12 (a)	0.90	0.082 (a)	0.011	28.2 (a)	0.94
Dry Forest								
Montane Forest	27.69 (a)	0.78	-22.84 (a)	0.57	0.049 (a,b,c)	0.012	25.51 (a)	0.48
Wet Forest	27.45 (a)	1.27	-25.99 (a)	0.59	0.027 (c)	0.006	25.85 (a)	0.53
Dry Forest	26.92 (a)	0.90	-22.72 (a)	1.16	0.058 (a,b,c)	0.013	26.29 (a)	0.52
Source Population x Distance								
5								
Montane Forest	28.78 (b)	0.49	-28.54 (a)	1.84	0.050 (f,g)	0.003	16.97 (f)	0.56
Wet Forest	34.40 (a)	0.43	-28.65 (a)	0.47	0.072 (e,f,g)	0.006	23.52 (e)	0.41

Dry Forest	32.23 (a)	1.41	-29.25 (a)	1.22	0.045 (g)	0.004	23.19 (e)	0.33
10								
Montane Forest	22.36 (c,d)	0.52	-24.75 (a)	0.68	0.119 (c,d)	0.009	15.11 (f)	1.26
Wet Forest	27.72 (b)	0.42	-23.09 (a)	0.47	0.086 (d,e,f)	0.006	22.98 (e)	0.19
Dry Forest	29.12 (b)	0.46	-23.72 (a)	0.44	0.075 (e,f,g)	0.005	22.48 (e)	0.16
20								
Montane Forest	14.25 (f)	0.89	-19.71 (a)	1.18	0.155 (b,c)	0.012	28.63 (c,d)	0.35
Wet Forest	19.53 (d,e)	0.57	-18.4 (a)	0.52	0.166 (b)	0.011	33.96 (a)	1.04
Dry Forest	24.38 (c)	0.56	-21 (a)	0.46	0.114 (d)	0.006	29.52 (b,c,d)	0.22
40								
Montane Forest	15.18 (e,f)	0.59	-20.04 (a)	0.72	0.104 (c,d,e)	0.005	26.05 (d,e)	0.43
Wet Forest	14.08 (f)	0.51	-15.64 (a)	0.38	0.191 (a,b)	0.010	32.21 (a,b)	0.94
Dry Forest	18.61 (d,e)	0.61	-19.48 (a)	0.67	0.223 (a)	0.025	30.47 (b,c)	0.60
Source Population x Distance								
5								
Montane Forest	33.14 (a)	0.57	-28.49 (a)	0.30	0.060 (f,g)	0.003	21.3 (a)	0.54
Wet Forest	32.50 (a)	1.55	-30.43 (a)	1.39	0.048 (g)	0.005	21.54 (a)	0.63
Dry Forest	32.03 (a)	0.70	-27.62 (a)	1.40	0.059 (f,g)	0.007	22.44 (a)	0.55
10								
Montane Forest	26.59 (a)	0.56	-22.11 (a)	0.29	0.118 (c,d)	0.006	20.88 (a)	0.77
Wet Forest	27.58 (a)	0.55	-25.53 (a)	0.39	0.076 (e,f,g)	0.005	20.83 (a)	0.77
Dry Forest	26.44 (a)	0.73	-23.69 (a)	0.68	0.086 (d,e,f)	0.008	20.43 (a)	0.79
20								
Montane Forest	19.69 (a)	1.02	-18.29 (a)	0.44	0.165 (a,b)	0.011	30.44 (a)	0.63
Wet Forest	21.05 (a)	0.78	-21.21 (a)	1.02	0.123 (c,d)	0.008	30.43 (a)	0.76
Dry Forest	18.72 (a)	0.85	-19.73 (a)	0.56	0.147 (c,d)	0.010	31.28 (a)	0.74
40								
Montane Forest	16.45 (a)	0.63	-16.9 (a)	0.53	0.198 (a)	0.021	29.73 (a)	0.83
Wet Forest	16.81 (a)	0.58	-19.88 (a)	0.54	0.118 (c,d,e)	0.012	30.1 (a)	0.90
Dry Forest	14.89 (a)	0.90	-17.32 (a)	0.90	0.206 (a)	0.022	30.8 (a)	1.00

Table 2.S5: Results for the four measurements of degradation of female full songs for all main effects and two-way interactions. Values presented are mean ± standard error. Results of post-hoc test are presented in brackets; within each separate section of the table, values are significantly different from each other are exhibit a different letter. Whereas the main text focuses on analyses of playback of isolated elements of wren songs, this analysis focuses on playback of entire songs.

Female Full Songs	**Signal-to-Noise Ratio**		**Tail-to-Signal Ratio**		**Blur Ratio**		**Excess Attenuation**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Playback Site								
Montane Forest	31.43 (a)	0.68	-27.47 (a)	0.48	0.093 (a)	0.006	18.25 (a)	0.72
Wet Forest	34.14 (b)	0.63	-26.55 (a)	0.41	0.082 (a,b)	0.006	23.46 (b)	0.49
Dry Forest	37.98 (c)	0.54	-29.69 (b)	0.43	0.059 (b)	0.006	23.47 (b)	0.35
Source Population								
Montane Forest	29.16 (a)	0.70	-22.15 (a)	0.41	0.061 (a)	0.005	22.61 (a)	0.54
Wet Forest	27.87 (a)	0.60	-22.50 (a)	0.39	0.087 (a)	0.006	20.36 (b)	0.51
Dry Forest	26.44 (a)	0.66	-22.88 (a)	0.52	0.086 (a)	0.006	22.22 (a,b)	0.53
Distance								
5	34.51 (a)	0.45	-27.96 (a)	0.35	0.078 (a)	0.003	21.73 (a)	0.35
10	29.06 (b)	0.56	-22.93 (b)	0.34	0.096 (b)	0.004	19.61 (b)	0.41
20	22.42 (c)	0.65	-18.94 (c)	0.34	0.159 (c)	0.007	29.85 (c)	0.56
40	19.54 (d)	0.54	-17.16 (c)	0.38	0.174 (c)	0.010	30.26 (c)	0.46
Playback Site x Source Population								
Montane Forest								
Montane Forest	23.77 (a)	1.14	-22.09 (a)	0.68	0.094 (a)	0.008	22.57 (a)	1.28
Wet Forest	24.58 (a)	1.16	-22.22 (a)	0.70	0.133 (a)	0.008	20.37 (a)	1.23
Dry Forest	21.19 (a)	1.19	-22.94 (a)	1.10	0.149 (a)	0.011	21.79 (a)	1.27
Wet Forest								
Montane Forest	29.58 (a)	1.17	-22.29 (a)	0.60	0.075 (a)	0.007	26.47 (a)	0.86
Wet Forest	26.57 (a)	0.98	-22.62 (a)	0.69	0.141 (a)	0.011	25.11 (a)	0.85
Dry Forest	26.41 (a)	1.10	-21.86 (a)	0.83	0.134 (a)	0.011	26.64 (a)	0.86
Dry Forest								
Montane Forest	32.62 (a)	1.04	-22.09 (a)	0.77	0.086 (a)	0.010	26.49 (a)	0.66
Wet Forest	31.16 (a)	0.84	-22.61 (a)	0.64	0.139 (a)	0.011	25.04 (a)	0.55
Dry Forest	29.97 (a)	0.90	-23.66 (a)	0.82	0.131 (a)	0.008	26.81 (a)	0.60
Playback Site x Distance								
5								
Montane Forest	31.43 (b)	0.54	-27.47 (d,e)	0.81	0.093 (b,c,d)	0.007	18.25 (e)	0.86
Wet Forest	34.14 (b)	0.69	-26.55 (d)	0.50	0.082 (d)	0.005	23.46 (c,d)	0.45

Dry Forest	37.98 (a)	0.71	-29.69 (e)	0.47	0.059 (d)	0.004	23.47 (c,d)	0.32
10								
Montane Forest	27.50 (c,d)	0.51	-23.53 (c)	0.59	0.088 (c)	0.005	14.76 (f)	1.17
Wet Forest	26.21 (c,d)	1.00	-21.93 (b,c)	0.57	0.104 (b,c)	0.008	22.69 (c,d)	0.25
Dry Forest	33.48 (b)	0.78	-23.51 (c)	0.57	0.097 (c)	0.007	21.36 (d,e)	0.19
20								
Montane Forest	15.41 (g)	0.68	-18.83 (a)	0.69	0.173 (a)	0.010	27.69 (b)	1.05
Wet Forest	24.02 (d,e)	0.94	-18.85 (a)	0.69	0.149 (a)	0.017	33.01 (a)	1.43
Dry Forest	27.83 (c)	0.74	-19.10 (a,b)	0.45	0.154 (a,b)	0.012	30.09 (a,b)	0.44
40								
Montane Forest	17.38 (g)	0.56	-18.89 (a,b)	0.70	0.143 (a,b)	0.014	26.69 (b,c)	0.40
Wet Forest	18.61 (f,g)	1.13	-16.87 (a)	0.69	0.188 (a)	0.021	31.30 (a,b)	1.03
Dry Forest	22.64 (e,f)	0.67	-16.61 (a)	0.56	0.190 (a)	0.015	31.51 (a)	0.51
Source Population x Distance								
5								
Montane Forest	35.32 (a)	0.94	-27.37 (a)	0.41	0.060 (a)	0.006	23.00 (a)	0.66
Wet Forest	35.73 (a)	0.56	-28.15 (a)	0.34	0.087 (a)	0.006	20.80 (a)	0.55
Dry Forest	33.65 (a)	0.77	-28.35 (a)	0.89	0.086 (a)	0.005	22.68 (a)	0.57
10								
Montane Forest	30.59 (a)	1.19	-22.66 (a)	0.45	0.072 (a)	0.006	20.47 (a)	0.73
Wet Forest	29.09 (a)	0.72	-23.15 (a)	0.33	0.108 (a)	0.008	19.35 (a)	0.73
Dry Forest	28.14 (a)	0.94	-22.98 (a)	0.86	0.109 (a)	0.006	20.76 (a)	0.69
20								
Montane Forest	24.01 (a)	1.25	-18.52 (a)	0.52	0.112 (a)	0.011	30.69 (a)	0.96
Wet Forest	22.72 (a)	0.99	-19.03 (a)	0.56	0.174 (a)	0.011	28.57 (a)	0.92
Dry Forest	20.71 (a)	1.10	-19.25 (a)	0.69	0.154 (a)	0.012	30.49 (a)	1.01
40								
Montane Forest	22.77 (a)	1.22	-16.80 (a)	0.90	0.143 (a)	0.018	30.68 (a)	0.83
Wet Forest	19.73 (a)	0.58	-16.72 (a)	0.36	0.188 (a)	0.014	29.58 (a)	0.81
Dry Forest	18.65 (a)	0.85	-18.08 (a)	0.72	0.189 (a)	0.012	31.07 (a)	0.69

Table 2.S6: Main effects for the linear mixed models analyzing comparisons of background noise during male (left) and female (right) full songs.

	Male Songs			Female Songs		
	df	**F**	**p**	**df**	**F**	**p**
Model	23	29.3	**<0.001**	12	20.59	**<0.001**
Playback Site	2	5.32	**0.006**	2	2.72	0.069
Source Population	2	0.02	0.98	2	0.04	0.965
Distance	3	125.05	**<0.001**	3	189.64	**<0.001**

Table 2.S7: Results for background noise (in dB) during transmission of male and female full songs and song elements for all main effects. Values presented are mean ± standard error. Results of post-hoc test are presented in brackets; within each separate section of the table, values are significantly different from each other are exhibit a different letter.

	Male Song		Female Song		Male Elements		Female Elements	
	Mean (dB)	**SE**	**Mean (dB)**	**SE**	**Mean (dB)**	**SE**	**Mean (dB)**	**SE**
Playback Site								
Montane Forest	36.71 (a)	9.53	81.47 (a)	8.97	29.54 (a)	6.02	26.70 (a)	5.88
Wet Forest	8.15 (b)	5.60	35.36 (b)	4.57	7.26 (b)	2.61	6.81 (b)	2.13
Dry Forest	7.93 (b)	3.90	26.54 (b)	3.63	6.38 (b)	2.12	4.42 (b)	1.25
Source Population								
Montane Forest	53.40 (a)	7.37	48.41 (a)	6.89	38.77 (a)	3.73	36.92 (a)	3.86
Wet Forest	47.27 (a)	6.69	43.81 (a)	5.77	36.51 (a)	3.36	31.64 (a)	2.73
Dry Forest	52.13 (a)	7.36	39.32 (a)	5.74	44.31 (a)	4.37	43.07 (a)	4.19
Distance								
5	17.60 (c)	2.21	13.51 (a)	1.59	14.39 (c)	1.22	12.65 (a)	0.89
10	28.20 (c)	5.15	19.87 (a)	3.29	21.40 (c)	2.59	18.09 (a)	1.82
20	67.40 (b)	8.12	58.44 (b)	7.45	52.36 (b)	4.79	49.21 (b)	4.51
40	127.45 (a)	8.04	94.36 (c)	7.38	98.26 (a)	5.26	90.43 (c)	5.15
Element Type								
Introductory Elements	NA		NA		38.97 (a)	3.81	31.79 (a)	2.93
Trill Elements	NA		NA		52.18 (a)	4.64	52.13 (a)	5.15
Terminal Elements	NA		NA		28.37 (a)	2.61	28.59 (a)	2.19

Chapter 3: Drift influences the evolution of male and female songs in a tropical songbird*

*This work is the outcome of joint research with D. Heath, R. Walter, M. Mark, and D. Mennill

Chapter Summary

Given the important role that animal vocalizations play in mate attraction and resource defence, variation in animal acoustic signals may feature prominantly in speciation. Research on the link between acoustic differences and genetic divergence expands our knowledge of the evolutionary implications of acoustic variation. Most studies, however, have focused on the acoustic traits of male songbirds living in the temperate zone. In contrast to temperate ecosystems, songs are often produced by both sexes in the tropics; therefore tropical animals offer a unique system for examining the evolution of acoustic signals in females, and for comparing differences between sexes. In this study we quantified patterns of acoustic variation in Rufous-and-white Wrens (*Thryophilus rufalbus*) from five populations along a 500 km transect in Central America. We examine whether male and female songs evolve differently by comparing the role that acoustic adaptation, cultural drift, dispersal, and genetic drift have played in shaping acoustic divergence. We quantified acoustic variation using fine-scale structural measurements of male and female songs, and genetic variation using both biparentally-inherited markers (DNA microsatellites) and maternally-inherited markers (mitochondrial DNA sequences). We found that males and females showed considerable acoustic and genetic structure among populations. Acoustic distance was correlated with both genetic distance and geographic distance, but when we controlled for both distances using partial Mantel tests, acoustic distance was correlated with neither. Our results imply that cultural drift has a greater influence on acoustic divergence than acoustic adaptation, dispersal, or genetic drift. Overall, our results provide greater insight into variation of male and female acoustic signals and the role of drift in the phenotypic and genetic evolution of tropical animals.

Introduction

Variation in the acoustic signals of animals can have profound evolutionary implications (Boughman, 2002). Acoustic signals play an important role in attracting mates and defending resources (Bradbury and Vehrencamp, 2011), and therefore changes in acoustic structure may promote reproductive isolation between populations (Jones, 1997; Irwin *et al*., 2001; Lemmon, 2009). Given the important role that acoustic signals may play in speciation, questions remain about the forces that drive the evolution of acoustic signals (Wilkins *et al*., 2013). Do acoustic signals evolve in unison with genetic drift? Or do acoustic signals evolve independently of biological evolution as a result of selection (MacDougall-Shackleton and MacDougall-Shackleton, 2001; Wright *et al*., 2005; Prohle *et al*., 2006; Campbell *et al*., 2010; Clegg and Phillimore, 2010; González *et* al., 2011)?

Ecological features influence acoustic variation (Burney and Brumfield, 2009; Wilkins *et al*., 2013), and animal signals vary with habitat structure (Hunter and Krebs, 1979; Handford and Lougheed, 1991; Slabbekoorn and Smith, 2002), ambient noise (Hanna *et al*., 2013; Mockford *et* al., 2013), and climate (Forrest, 1994; Brumm and Naguib, 2009). Similarly, ecological barriers such as habitat gaps (Glor and Warren, 2011), physical barriers (e.g. mountain passes or rivers; Pérez-Emán, 2005; Castoe *et al*., 2009), and climatic differences (Pilot *et al*., 2006) can influence genetic differentiation. Given the role that ecology plays in the evolutionary process, combining ecological data with genetic and acoustic data will provide greater insight into evolutionary patterns (Manel *et al.,* 2003; Kozak *et al*., 2008).

The songs of birds vary geographically, although most research on this topic focuses on temperate-breeding birds where only male birds sing (primarily in the North Temperate Zone; Podos and Warren, 2007). This is problematic given that 80% of all bird species breed at tropical latitudes, and that temperate and tropical birds often exhibit very different life history traits (Stutchbury and

Morton, 2008; Martin, 2015). For example, whereas many temperate species undergo annual long-distance migrations between their breeding and wintering grounds, the majority of tropical birds show strong philopatry and inhabit their breeding territories year round (Stutchbury and Morton, 2008). Additionally, tropical birds also exhibit different vocal behaviours from their temperate counterparts. For example, female song and male-female duets are widespread in the tropics, whereas these behaviours are rare or absent in the North Temperate Zone (Slater and Mann, 2004). Given the historical focus on temperate song, much less is known about female song (Langmore, 1998), in spite of the fact that female song is the ancestral trait for birds (Odom *et al.,* 2014). For these reasons, more research is needed on female song at both local scales (e.g. Mennill and Vehrencamp, 2005; Logue, 2007) and broad geographical scales (e.g. Mennill and Rogers, 2006), to better quantify patterns of variation in female songs.

In this study we examine acoustic variation in Rufous-and-white Wrens (*Thryophilus rufalbus*). This species is a year-round resident of the tropics and has a broad distribution that extends from southern Mexico, through Central America, and into Colombia and Venezuela. Both males and females sing in this species, songs show structural differences between sexes, and both males and females possess repertoires of song types (Mennill and Vehrencamp, 2005). Song-learning has not been studied in this species, but our observations on acoustic similarity suggest that males learn songs primarily from males, and females learn songs from females, as has been demonstrated in other duetting species (Mennill and Rogers, 2006; Evans and Kleindorfer, 2016).

To investigate the factors that contribute to acoustic variation in male and female Rufous-and-white Wrens, we studied five populations along a 500 km transect in Central America. Our study sites vary in habitat structure and climate, allowing us to also examine the role that ecology plays in shaping acoustic variation. Additionally, ongoing analyses of both DNA microsatellite and mtDNA genetic patterns indicate substantial population structure among our study sites (see supplementary

methods and results; Figure 3.S1). While DNA microsatellite and mtDNA patterns show some similarities, there are also some differences, and therefore we incorporated both DNA microsatellite and mtDNA markers to determine if acoustic variation reflects contemporary (microsatellites) or historical (mtDNA) genetic patterns. Given that tropical animals display strong philopatry and limited dispersal, gene flow is potentially limited between populations, and therefore songs may be distinct at the population level (Salisbury *et al*., 2012). We calculated acoustic distance, ecological distance, geographical distance, and genetic distance (using both biparentally-inherited DNA microsatellite markers and maternally-inherited mitochondrial DNA sequences) among populations. By comparing these four different sources of variation, we examined whether acoustic differences between populations arise as a result of acoustic adaptation, dispersal, cultural drift, or genetic drift (Table 3.1). For example, a significant positive relationship between acoustic distance and genetic distance would suggest that acoustic variation is influenced by genetic drift, whereas a negative or non-significant relationship would indicate that acoustic variation is more heavily influenced by selection (Ruegg *et al*., 2006). Given that both male and female Rufous-and-white Wrens sing, we also examine whether males and females show similar patterns of acoustic divergence.

Table 3.1: Discription of the five hypotheses tested to determine which factors play an important role in the evolution of male and female Rufous-and-white wren songs.

Hypothesis	Expected predictions of the hypothesis
Acoustic Adaptation Hypothesis	If acoustic divergance between population arises as a result of acoustic adaptation, we would expect to see a strong correlation between acoustic distance and ecological distance.
Cultural Drift Hypothesis	If acoustic divergence between populations is associated with different cultural selection patterns at each population, we would expect to see no correlation between acoustic distance and eclogical distance, microsatellite genetic distance, geographic distance, and mtDNA genetic distance.
Dispersal Hypothesis	If acoustic divergence between populations is associated with different cultural selection patterns at each population, we would expect to see a strong correlation between acoustic distance and geographic distance.
Genetic Drift Hypothesis (microsatellites)	If acoustic divergence between populations arises as a result of contemporary patterns of gene flow and genetic variation, we would expect to see a strong correlation between acoustic distance and microsatellite genetic distance.
Genetic Drift Hypothesis (mtDNA)	If acoustic divergence between populations reflects patterns of historical isolation or gene flow, then we would expect to see a strong correlation between mtDNA genetic distance and acoustic distance.

Methods

We studied Rufous-and-white Wrens in five populations (Figure 3.1): four populations in Costa Rica (Sector Santa Rosa of the Guanacaste Conservation Area: 10.85 °N, 85.60 °W; Sector Rincón de la Vieja of the Guanacaste Conservation Area: 10.78 °N, 85.35° W; University of Georgia Campus in the San Luis Valley near Monteverde: 10.28 °N, 84.79 °W; Central Valley: 9.90 °N, 84.25 °W) and one population in Nicaragua (Reserva Miraflora: 13.27 °N, 86.31 °W). We monitored birds in Costa Rica from 2012-2014, and birds in Nicaragua from 2004-2008. The Nicaragua site is located on the Pacific slope of Nicaragua and the vegetation at this site is Pre-montane Moist Forest (following Holdridge life zone classification; Holdridge, 1967; Mark, 2009). The vegetation at Santa

Rosa site is Tropical Dry Forest (Holdridge, 1967); the understory here is relatively open and this area experiences a dry season from November to April, followed by an intense rainy season from May to October. The vegetation at Rincón de la Vieja is representative of a premontane Moist-Wet Forest (Holdridge, 1967). The vegetation at Monteverde is Lower Montane Wet Forest. Finally, forest habitat in the Central Valley is also representative of a Moist Forest (Holdridge, 1967); however, the population we studied lies in an urban area where the forest is quite fragmented and the small patches of forest are surrounded by coffee plantations, agriculture, and housing areas. In each population, we captured birds using mist-nets and banded each individual with a unique band combination that included three colour bands and one numbered aluminum band. From each bird we collected a small blood sample (~100 μl) from the brachial vein, and stored blood samples in 95% ethanol or Queen's Lysis Buffer (Seutin *et al*., 1991). Individuals were sexed based on the presence of a brood patch (females) and by singing behaviour (sexes can be distinguished based on fine-structural differences in songs; Mennill and Vehrencamp 2005).

Acoustic measurements

We recorded birds between April and July of each year, a time of year when vocal output is high for this species (Topp and Mennill, 2007). Most of our recordings (60%) were collected using focal recording methods, where we followed each bird around its territory for several hours during the morning (between 0445h and 1100h) and confirmed the bird's identity by observing its leg bands. Focal recordings were collected using a solid-state digital recorder (Marantz PMD-660; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70). We supplemented these recordings with recordings from automated recorders (Song Meter SM2, Wildlife Acoustics Inc., Concord, Massachusetts, USA; sampling frequency: 44.1 KHz; full equipment details in Mennill et al. 2012). We placed these recorders near the centre of the territories of each focal pair, usually within 10m of the pair's nest. We confirmed that the songs collected on these

recorders were those of the intended birds by re-sighting the focal individuals in their territory after automated recording sessions, and by matching the songs collected by the automated recorders to the songs collected during focal recordings (see Harris *et al.*, 2016 for further details).

We annotated all audio files using SYRINX-PC sound analysis software (J. Burt, Seattle, Washington, USA). For each male and female we built a song library of all the songs that each bird sang. Once we had created a song library for each bird, we selected songs that had a high signal-to-noise ratio and collected fine-structural measurements from these songs. For each bird we measured up to five exemplars of each song type (males: average of 2.63 exemplars, range 1-5; females: average of 2.07 exemplars, range 1-5), and calculated a mean measurement for each song type for each individual. Whenever possible, we included songs from multiple recording sessions, measuring no more than three exemplars of each song type per recording.

To quantify geographic variation in the songs of male and female Rufous-and-white Wrens we measured eight different temporal and spectral features of their songs (Figure 3.2): (i) duration of the song (s), (ii) duration of the terminal syllable (s), (iii) duration of all the pauses in the song (s; we considered a pause as the space between one syllable and the next syllable), (iv) dominant frequency of the trill (Hz), (v) minimum frequency of the song (Hz), (vi) maximum frequency of the song (Hz), (vii) number of syllables in each song, and (viii) the bandwidth of the terminal syllable (Hz; calculated by subtracting the minimum frequency of the terminal syllable from the maximum frequency of the terminal syllable). We used the automated parameter measurements tool in AviSoft-SASLab Pro (version: 5.2.04; R. Sprecht; Berlin, Germany) to measure the fine-structural measurements of all songs, thereby minimizing the subjectivity in collecting these measurements. Songs were resampled to 8000 Hz, which allowed maximal spectral resolution (the maximum frequency of Rufous-and-white Wren songs in this dataset was less than 4000 Hz). For each song we created a spectrogram, with an effective resolution of 8Hz and 4 ms (settings: transform size: 1024

Hz; overlap: 96.86%; window: Hamming). We used a high-pass filter of 500 Hz to remove any low-frequency background noise from the sound files. The measurements used for all statistical analysis represent population means for each song type (males: an average of 4.88 individuals per song type, range = 1-28; females: 3.81 individuals per song type; range = 1-17). We obtained population means for each song type by calculating the individual means for each song type in an individual's repertoire (see above). Overall we measured 1669 male songs representing 134 song types from 91 individuals in five populations and 670 female songs representing 79 song types from 71 individuals in four Costa Rican populations. We recorded fewer than ten female songs in our Nicaragua population, and all but two were of poor quality and we could not rigorously quantify variation in female songs in this population.

Genetic analysis

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega), and genotyped 211 individuals (129 males and 81 females plus 1 individual from Nicaragua whose sex was as unknown) at 10 microsatellite loci. We used four previously designed microsatellite primer sets *Th-Pl 14, Th-Pl 20, Th-Pl 30* (Brar *et al*., 2007), *RWWR 2c* (Hermann Mays personal communication), and developed six new microsatellite primer sets (*Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25;* Table 3.S1) following the microsatellite enrichment procedure detailed in Walter *et al*. (2007). All PCR reactions were conducted in 12.5 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 1.25 μL of 10x PCR buffer (Applied Biosystems), 0.5 μL of $MgCl_2$ (2.5 mM), 0.45 μl of dNTPs (0.2 mM), 0.05 μl of bovine serum albumin, and 0.5 U of Taq (Genscript, Applied Biosystems). For the primer sets *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25,* and *RWWR 2c,* we included 1 μM each of the tel-forward, reverse, and M13 dye-labeled primer (GTAAAACGACGGCCAGT). For the remaining three primer sets (*Th-Pl 14, Th-Pl 20,* and *Th-Pl 30*) we used 1 μM each of the forward primer and the IR-dye labeled reverse primer. PCR conditions for *Th-*

Pl 14, Th-Pl 20, and *Th-Pl 30* followed those described in Douglas *et al*. (2012), while for the remaining primer sets we used the following PCR conditions: one cycle of 94.0°C for 2 minutes, followed by 34 cycles of 94.0 °C for 10 seconds, 50.0°C for 10 seconds, 72.0°C for 30 seconds, followed by a final extension cycle of 72.0°C for 90 seconds, although for the primer set *Tru* 24 we increased the annealing temperature (T_2) to 54.0°C to eliminate stuttering. PCR products were visualized on a 6% acrylamide gel on a Licor 4300 DNA analyzer. To ensure consistent sizing and scoring across gels, we ran controls with known size standards on each run. Allele sizes were scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

We genotyped 57 individuals at the NADH dehydrogenase 2 mitochondrial gene (ND2). We amplified ND2 sequences using previously designed primers (forward primer L5215; TATCGGGCCCATACCCCGAAAAT; Hackett, 1996; reverse primer H1064 CTTTGAAGGCCTTCGGTTTA; Drovetski *et al*., 2004). All PCR reactions were conducted in 25 μl reactions with 1 μl of genomic DNA. PCR cocktails contained 2.5 μl of 10x PCR Buffer (Applied Biosystems), 1.0 μl of $MgCl_2$ (2.5 mM), 0.9 μl of DNTP (0.2 mM), 1μM each of the forward and reverse primer, and 1.0 U of taq (Genscript, Applied Biosystems). PCR thermocycler conditions used the following conditions: one cycle of 94.0 °C for three minutes, followed by 35 cycles of 94.0 °C for 40 seconds, 50.0 °C for 40 seconds, 72 °C for one minute, followed by a final extension cycle of 72 °C for three minutes. PCR amplicons were sequenced with the forward primers at the McGill University and Génome Quebec Innovation Center. Sequences were aligned and trimmed to their respective lengths using Mega 5.0 (Tamura *et al*., 2007).

For our microsatellite dataset, we tested for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium each population x loci combination in GenePop version 4.0.10 (Raymond and Rousset, 1995), and corrected for multiple tests using sequential Bonferroni corrections (Rice, 1989). We calculated allelic richness (A_R), observed heterozygosity (H_O), and

expected heterozygosity (H_E) using FSTAT version 2.9.2.3 (Goudet, 1995). For our mtDNA dataset, we calculated nucleotide diversity (π), and haplotype diversity (H_d) for each population using DNAsp version 5.0 (Librado and Rozas, 2009).

We used microsatellite and mtDNA markers to assess genetic population structure at different temporal scales. Given that microsatellites evolve very quickly (1×10^{-3}-10^{-4}; Weber and Wong, 1993), this allowed us to examine recent genetic changes (Primmer *et al.,* 1996), whereas mtDNA evolves much slower (approximately 2.3%/million years) and therefore allowed us to quantify historical genetic changes (Smith and Klicka, 2010). We calculated pairwise F_{ST} values (microsatellites) using FSTAT, and pairwise θ_{ST} values (mtDNA) using ARLEQUIN version 3.11 (Excoffier *et al*., 2005); we tested for significant deviations from zero using 10,000 permutations for both sets of comparisons. All pairwise tests were corrected using sequential Bonferroni tests.

Gene flow and dispersal patterns

To better determine the relationship between genetic differentiation and song differentiation we estimated rates of gene flow between populations using our microsatellite dataset in the program BAYESASS+ (version 3.0; Wilson and Rannala, 2003). We used BAYEASS to estimate contemporary gene flow, as the values are indicative of gene flow over the last few generations (Wilson and Rannala, 2003). Given that learned traits like song can evolve quickly over short time periods (Payne, 1996), we estimated gene flow with our microsatellite dataset only, because mtDNA estimates depict rates of female dispersal and gene flow only over a longer time frame, and thus are less useful for the level of song analysis. We ran ten replicates for 10 000 000 Monte Carlo Markov Chains with a burn-in of 1 000 000; we altered mixing parameters to ensure that allele frequency, inbreeding coefficients, and migration rates fell within the 20 to 60% acceptance rate suggested by Wilson and Rannala (2003). Lastly we calculated Bayesian deviance values (Spiegelhalter *et al*., 2002) in R 3.2.3 (using the script provided in Meirmans, 2014) to

determine the best-fit run; results presented for BAYEASASS are from this best-fit run (Chiucchi and Gibbs, 2010).

In addition to characterizing gene flow, we also used our microsatellite dataset to characterize dispersal patterns. Female-biased dispersal is common in birds (Greenwood, 1980), and given that dispersal is predicted to influence acoustic variation (Lynch, 1996, Wright and Wilkinson, 2001), we used the sex-biased dispersal option in FSTAT to determine whether male and female Rufous-and-white Wrens exhibit differences in dispersal. For our analysis, we compared corrected assignment indices and variance of corrected assignment indices between sexes to determine if Rufous-and-white Wrens show patterns of sex-biased dispersal. Assignment indices are used to detect immigrants within populations; the more common a genotype is, the higher (and more positive) the value is, whereas individuals with low (and more negative) values are more likely to be immigrants. Therefore, the sex with the lowest assignment index value is considered the more dispersive sex (Favre *et al*., 1997; Mossman and Waser, 1999). Furthermore, the more dispersive sex is expected to have a larger variance than the other sex, because they will have a combination of both resident and immigrant birds. To compare whether sexes exhibited similar patterns of dispersal, we used two-sided assignment probability and variance tests.

Ecological data

Integrating ecological data into evolutionary studies (Kozak *et* al., 2008) has helped to further explore the relationship between phenotypic and genetic patterns in other animals (Ruegg *et al.*, 2006; Wang and Summers, 2010). Given that habitat is known to influence the evolution of animal sounds (Morton, 1975), we incorporated environmental data to better characterize the factors that contribute to song variation in Rufous-and-white Wrens. Habitat may have a strong influence on the songs of non-migratory animals, such as Rufous-and-white Wrens, given that they are found year-round on their territories (Mennill and Vehrencamp, 2005). We downloaded climate

data from the WorldClim database (http://worldclim.org) and extracted data using QGIS. The extracted climate data include mean values from over 50 years (1950-2004; Hijmins *et* al., 2005) and with a spatial resolution of ~1 km (Table 3.S2). Many of the 20 variables were intercorrelated (Pearsons' r >0.70), and therefore we quantified habitat variation using four uncorrelated variables: (i) precipitation of driest quarter (mm), (ii) precipitation of coldest quarter (mm), (iii) temperature annual range (°C), and (iv) mean temperature of driest quarter (°C). Additionally, we included a quantitative variable to characterize low frequency background noise (0-1 kHz), given that biological, abiotic, and urban noise sources can influence the evolution of acoustic signals (Slabbekoorn and Peet, 2003; Hanna *et al*., 2011; Luther and Derryberry, 2012; see Chapter 2). Many animals shift the frequency of their vocalizations to avoid having their vocalizations masked by background noise, and acoustic differences could reflect noise differences between sites (Slabbekoorn and Peet, 2003). We focused on this particular area of noise (between 0 and 1 KHz), because many of the introductory and trill syllables of male and female Rufous-and-white Wrens are produced between 500 Hz and 1 kHz, and this frequency is expected to experience heavy overlapping by background noise (Slabbekoorn, 2004). We quantified sites as having low (score=1), moderate (score=2), or high (score=3) background noise, by visually inspecting spectrograms of focal recordings (Table S2). To produce a single ecological distance between the five sites, we performed a principal component analysis on our five variables using direct oblimin rotation, because this method allows for correlations between components, and retained the first three principal components with Eigenvalues above 1.0. We then calculated the Euclidean distances between population means of the first three principal components to create an ecological dissimilarity matrix.

Statistical analysis

Acoustic variation: We analyzed acoustic data using two methods. First, we used a Discriminant Function Analysis on male and female songs separately to evaluate whether source

populations (the site where the bird was recorded) were distinguishable based on the eight fine-structural measurements of songs (see supplemental Table 3.S3 for factor loadings). For this analysis we used the leave-one-out classification approach, and we report the percentage of songs correctly assigned to the correct group using the cross validation approach as employed in SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). We used Chi-square tests to evaluate whether our discriminant function analysis successfully assigned songs to the correct population, at a level that exceeded chance. Second, we used a Multivariate Analysis of Variance (MANOVA) to examine if acoustic variables were significantly different among populations. Prior to analysis, acoustic variables were tested for intercorrelations using a Pearson correlation analysis; no correlations (r) exceeded 0.7 and therefore all variables were included in both analyses (Ruegg *et al*., 2006). We also checked for normality using Shapiro-Wilks tests and by visually inspecting the Q-plots of the residuals for each acoustic variable. Four acoustic variables for males (bandwidth of terminal syllable, length of terminal syllable, duration of all pauses in the song, and number of syllables) and three acoustic variables for females (bandwidth of terminal syllable, length of terminal syllable, and number of syllables) were log-transformed to improve normality.

Comparisons between acoustic, genetic, and ecological divergence: We used a causal modeling approach to test whether acoustic divergence in Rufous-and-white Wren songs is correlated with acoustic adaptation (ecological distance), dispersal (geographical distance), genetic drift (microsatellite or mtDNA genetic distance), or a combination of these factors (Summers and Wang, 2010). We used Mantel and partial Mantel tests in these analyses. Partial Mantel tests allowed us to test the effect of one variable on acoustic divergence, while controlling for the effects of other variables. We generated Euclidean distances for acoustic data from each population using the mean coordinates for the first two discriminant axes for each population. Ecological distance was calculated as described above. To calculate genetic divergence, we converted our microsatellite

F_{ST} values, and mitochondrial θ_{ST} values to a genetic distance using the formula (1- F_{ST})/F_{ST} (replacing F_{ST} in the formula with θ_{ST} for our mtDNA results). Geographic distance was measured as the straight-line distance between paired sites, and was calculated in GenALex. All Mantel tests and partial Mantel tests were performed in GENODIVE (Meirmens and van Tienderen, 2004) for 100 000 permutations.

Results

Acoustic variation: Males

We observed substantial variation in male songs among populations of Rufous-and-white Wrens. Discriminant analysis assigned songs to the correct population better than expected by chance (X^2=38.02, df=16, p=0.002; Table 3.2), yet only 36.6% of songs were assigned to the correct population. The Central Valley and Santa Rosa songs had the highest percentage of correct song assignment, whereas Nicaragua and Monteverde had the lowest percentage of correct song assignment.

We found significant differences in the fine-structural features of male songs between populations using multivariate analysis of variance (Wilks' lambda=0.42; $F_{451,32}$ =3.78, p<0.001, partial eta^2 = 0.20). Three fine-structural measurements showed significant differences between populations: duration of terminal syllables ($F_{129,4}$ = 12.96, p<0.001, partial eta^2=0.29), bandwidth of terminal syllables ($F_{129,4}$ =3.07, p=0.02, partial eta^2=0.09), and minimum frequency of songs ($F_{129,4}$ =3.45, p=0.01, partial eta^2=0.10; Table 3.3). Five of ten post-hoc pairwise comparisons were significant for the duration of terminal syllables; terminal syllables from Nicaragua (0.06$\pm$0.03 s) were significantly shorter than terminal syllables from the Central Valley (0.20$\pm$0.10 s), Monteverde (0.14$\pm$0.07 s), and Rincon (0.17$\pm$0.07 s); and Santa Rosa terminal syllables were significantly shorter (0.11$\pm$0.08 s) than terminal syllables from Rincon and the Central Valley. For the bandwidth of the terminal syllable, only one of ten pairwise comparisons was significantly different: terminal syllables

from Santa Rosa span a larger bandwidth (470±516 Hz) than at Rincon (179±320 Hz). Similarly only one of ten pairwise comparisons was significant for minimum frequency: Central Valley songs have a higher minimum frequency (803±13 Hz) than Rincon (735±14 Hz).

Acoustic variation: Females

We also observed substantial acoustic variation in the structure of female songs between four populations of Rufous-and-white Wren. Discriminant analysis assigned female songs to the correct population better than expected by chance (X^2=36.07, df=9, p<0.001; Table 3.2). Overall, 53.2% of female songs were assigned to the correct population; by comparison discriminant analysis correctly assigned more female songs than male songs to the correct population (36.3%). For females, Monteverde had the lowest percentage of songs correctly assigned (11.1%; two of 18 songs), while Santa Rosa had the highest percentage of songs correctly assigned (84.4%; 27 of 32 at Santa Rosa).

Multivariate analysis of variance demonstrated that songs vary significantly among populations (Wilks lambda=0.36, F=3.46, p<0.001, partial eta^2=0.29; Table 3.3). Three variables were significantly different among populations: terminal syllable bandwidth (F=13.62, p<0.001, partial eta^2=0.35), dominant frequency of the trill (F=2.98, p=0.04, partial eta^2=0.11), and minimum frequency of the song (F=5.68, p=0.001, partial eta^2=0.19). Post-hoc analyses revealed that the bandwidth of the terminal syllables from Santa Rosa was significantly different from all other populations ($p \leq 0.02$), as syllables from Santa Rosa cover a larger bandwidth (741±535 Hz) than terminal syllables from any of the other populations. Furthermore dominant frequency of the song is significantly different (p=0.048) between Santa Rosa (1029±25 Hz) and the Central valley (1100±32 Hz). Similarly, Santa Rosa (872±26) and Central Valley (1087±46) also showed significant differences for minimum frequency of the song (p=0.001)

Acoustic divergence

Pairwise Euclidean distances for male acoustic measurements ranged from 0.51 to 2.79 among populations (Table 3.4); Monteverde and Rincon had the most similar songs (0.51), while the Central Valley and Nicaragua had the most divergent songs (2.79). Interestingly, Santa Rosa songs were more similar to Monteverde and Central Valley songs (1.13 and 1.26 respectively) than they were to Rincon songs (1.80), despite the fact that Santa Rosa is closer to Rincon (28 km) than the other two populations (108 and 181 km). Euclidean distances for female acoustic measurements ranged from 0.72 to 2.41. As we observed for male acoustic distances, Monteverde and Rincon had the most similar songs, while the Central Valley and Santa Rosa had the least similar songs (2.41). Like male songs, Santa Rosa songs were more similar to Monteverde songs (1.59) than they were to Rincon songs (2.30).

Genetic variation

We genotyped 211 Rufous-and-white Wrens from five sample sites at ten microsatellite loci. The ten microsatellite loci showed high variability, containing between 2 and 35 alleles (average: 14.7±3.1; Table 3.5); three of fifty (6%) locus × population comparisons showed significant departures from HWE, while only one of two-hundred and twenty five (0.004%) locus × population comparisons showed significant linkage disequilibrium. Two of the three locus x population combinations that were not in HWE were found at Santa Rosa; to ensure that departures from HWE were not driving the observed patterns, we performed our analysis with all 10 loci and then repeated the analyses without the two loci that showed significant departures from HWE at Santa Rosa (*ThPl-14* and *ThPl-30*). We used the full microsatellite data set for all analyses, as removing these loci did not significantly change our results.

We sequenced the full ND2 gene (1041 bp) for 57 Rufous-and-white Wrens from five sites in Central America. We found 37 variable sites (31 of which were parsimony informative) and

identified 40 haplotypes. Both haplotype and nucleotide diversity were high (0.978 and 0.001 respectively), with Santa Rosa exhibiting higher haplotype and nucleotide diversity than all other populations (Table 3.5).

Pairwise comparisons of our microsatellite and mtDNA datasets suggest significant genetic differentiation among our five populations. Our genetic analyses reveal population structure among populations of Rufous-and-white Wrens in Central America; ten of ten F_{ST} pairwise comparisons and eight of ten θ_{ST} pairwise comparisons were significant (Table 3.6; F_{ST} values ranged from 0.03 to 0.10; θ_{ST} ranged from -0.04 to 0.81). The Nicaragua population was significantly different from all Costa Rican populations for both F_{ST} and θ_{ST} comparisons. Two pairwise comparisons (Monteverde versus Central Valley, and Santa Rosa versus Rincon) showed contrasting patterns between markers, and in both cases θ_{ST} pairwise comparisons were not significantly different for these two pairs of populations (0.02 and -0.04 respectively; Table 3.6).

Dispersal and gene flow

Our analyses show limited evidence of migration among populations of Rufous-and-white Wrens. The majority of estimates made using BAYESASS+ (15 of 20) were very low (<0.03), but only 1 of 20 comparisons were distinguishable from estimates generated with uninformative data, suggesting that dispersal events are difficult to detect in our dataset. Only our estimate of migration from Monteverde and the Central Valley fell outside of these confidence intervals. In this instance, estimates of migration between Monteverde and the Central Valley was 0.24; this suggests relatively high immigrant ancestry in the Central Valley from Monteverde.

Our comparison of assignment indices between males and females suggest that dispersal is female-biased. Females had a lower mean assignment index (assignment $index_{females}$=-0.27) than males did (assignment $index_{males}$=0.16), although this difference was not significant (p=0.47). We did, however, observe a significant relationship for differences in variance in assignment indices

(p=0.03); females had a higher variance (assignment index variance$_{females}$=20.67) than did males (assignment index variance$_{males}$=10.26). This pattern is consistent with the idea that females are the more dispersive sex, as is generally common in birds.

Ecological divergence

Pairwise Euclidean distance of ecological measurements, based on both WorldClim data and qualitative ambient noise measurements, show that Santa Rosa and Nicaragua are more different ecologically than the three other populations in our study; both of these sites receive less precipitation than the other three sites annually (average Euclidean distance was 2.28 and 2.34 respectively). The largest difference was observed between Santa Rosa and Monteverde (2.75); compared to Monteverde, conditions are much drier and hotter at Santa Rosa (precipitation values were higher at Monteverde than Santa Rosa for all both precipitation variables analyzed in our study). Rincon and Monteverde were our two most similar sites (0.66); while Monteverde is slightly wetter and cooler, annual temperature and precipitation are very similar between sites. Our urban site located in the Central Valley was most different from both Santa Rosa and Nicaragua (1.77 and 2.61 respectively), but was more similar to Rincon and Monteverde (0.77 and 1.31). This site received intermediate levels of precipitation during the dry season, but temperatures were slightly warmer here during the driest quarter (23.0°C) than all other sites except for Santa Rosa (24.8°C).

Causal modeling analyses

Male acoustic distance was significantly correlated with microsatellite genetic distance (r=0.67, p=0.04; Table 3.7) and geographic distance (r=0.76, p=0.02), but not ecological distance (r=0.55, p=0.10) or mtDNA distance (r=0.70, p=0.06). When we accounted for ecological distance, geographical distance, and genetic distance in our subsequent partial Mantel tests, acoustic distance was not correlated with geographic distance or microsatellite genetic distance. Female acoustic distance was not significantly correlated with genetic distance, ecological distance, or geographical

distance (Table 3.7). Taken together, these results support the hypothesis that cultural drift and cultural selection drive both male and female acoustic variation. Geographic distance was strongly correlated with both microsatellite genetic distance (r=0.67, p=0.01) and mtDNA genetic distance (r=0.86, p=0.02), indicating that genetic patterns fit an isolation-by-distance model.

Discussion

We explored the relationship between acoustic variation, ecological variation, and genetic variation in five populations of Rufous-and-white Wrens in Central America. We evaluated whether acoustic adaptation, dispersal, cultural drift, genetic drift, or these factors in concert influence acoustic divergence in male and female Rufous-and-white Wrens. Although genetic distance and geographic distance were correlated with acoustic distance, acoustic distance was not correlated with either variable when we controlled for each variable using partial Mantel tests. Therefore our results support the hypothesis that cultural drift or cultural selection drive acoustic variation in both male and female Rufous-and-white Wrens. While acoustic variation and genetic variation show similar patterns, cultural patterns seem to change independently of genetic changes. Overall, males and females showed similar patterns of acoustic divergence suggesting that similar evolutionary processes act on the evolution of male and female songs (Chapter 2). Acoustic patterns are more closely correlated with contemporary genetic patterns (microsatellites), than historical genetic patterns (mtDNA), further supporting the idea that cultural drift is an important driver in the evolution of both male and female songs. While we found weak support for acoustic adaptation, we discuss further below the role that habitat and environmental differences between populations may have on acoustic variation in Rufous-and-white Wrens.

Cultural drift is a key component of acoustic evolution, driving both temporal variation within populations and geographic variation (Lynch, 1996; Podos and Warren, 2007; Wright *et al*., 2008; Byers *et al*., 2010; Lin *et al*., 2014; Potvin and Clegg, 2015). In our study the majority of our

causal models were not significant, and therefore cultural drift and selection appear to be the main drivers of acoustic variation in male and female Rufous-and-white Wrens. Acoustic differences may arise between populations as a result of neutral song variation or reflect different selection pressures or mating preferences at each site (Podos and Warren, 2007; Collins *et al*., 2009). Neutral song variation occurs as a result of improvisation or improper song learning by young birds, where copying errors introduced during the song learning process may change song structure and drive cultural differences between populations (Lynch, 1996; Ellers and Slabbekoorn, 2003). With respect to cultural selection, individual mating preferences may not only influence changes in song structure, but also the song types that are used in populations (Cardoso and Atwell, 2011). For example, some tropical species follow duet codes when coordinating their songs to produce duets with their mates (Logue, 2006; Templeton *et al*., 2013a). In these species, males and females respond to each other's songs consistently with the same song types (Logue, 2007; Templeton *et al*., 2013b). The phenomenon of duetting may apply its own cultural pressures, if animals follow these duet codes. Individuals may learn specific song types because they are culturally selected or sexually selected for by mates.

Dispersal or limitation therein and ecological specialization are considered key drivers of speciation at tropical latitudes (Prohl *et al*., 2006; Claramunt *et al*., 2012; Salisbury *et al*., 2012; Smith *et al*., 2014); drift is likely to act in concert with dispersal, restricted gene flow, and ecological specialization as a strong driving force of both phenotypic and genetic divergence (Ellers and Slabbekoorn, 2003). Although genetic drift influences acoustic divergence in animals that do not learn their acoustic signals (e.g. Isler *et al.*, 2005; Campbell *et al*., 2010), there is little evidence to suggest that genetic drift and acoustic variation are linked in animals that learn their acoustic signals (Soha *et al*., 2004; Wright *et* al., 2005; Leader *et* al., 2008; Yoktan *et al.*, 2011; Ortiz-Ramírez *et al*., 2016; but see Baker *et al.*, 1982; MacDougall-Shackleton and MacDougall-Shackleton, 2001). In our

study, genetic and phenotypic patterns fit an isolation-by-distance model, and estimates of migration levels between populations were low. Therefore similarities between acoustic and genetic patterns likely reflect limited dispersal between populations, resulting in cultural drift and genetic drift shaping contemporary acoustic and genetic patterns (Andrew, 1962).

Ecological selection is an important driver of acoustic divergence. While previous studies have demonstrated ecological selection as an important driver of acoustic divergence, we found limited support for a relationship between ecological variables and acoustic structure, as would be predicted by the Acoustic Adaptation Hypothesis (Salbbekoorn and Smith, 2001; Ruegg *et al*., 2006; Caro *et al*., 2013; see Chapter 2). Despite this result, patterns of acoustic variation may still be influenced by ecological variation. For example, the habitat at the three populations in our third microsatellite cluster are similar based on climate data (Holdridge, 1967). The lowest male and female pairwise comparisons of acoustic distance were between these three sites, and these results may indicate that songs are adapted to these sites. Additionally, our ecological measurements may not accurately depict the ecological differences between sites, given that we only used four climate variables and one categorical noise level to characterize habitat and ecological differences between sites. Further ecological differences may be more difficult to detect, given that Rufous-and-white Wrens strictly inhabit forests (Stotz *et* al., 2007), and their songs are adapted for optimal transmission in forests (Barker *et al*., 2009, Chapter 2). By comparison, previous studies have compared acoustic variation among broadly distributed species that live in drastically different habitats (for example, open grasslands versus densely vegetated forested habitats; Handford and Lougheed, 1991).

While acoustic structure is often correlated with habitat structure (Boncoraglio and Saino, 2007), other factors like ambient noise, and acoustic competition with other species may influence acoustic structure (Handford and Lougheed, 1991; Dingle *et al*., 2008; Luther, 2009; Azar *et al*.,

2014; Hart *et al*, 2015). Previous work at three of the five sites used in this study (Santa Rosa, Rincon, and Monteverde), has demonstrated that ambient noise levels are significantly different among sites (Chapter 2). Furthermore the Central Valley site is located in the middle of a heavily populated urban area; animals are known to produce signals at a higher frequency in urban areas, so that their signals can be heard above traffic or other sources of anthropogenic noise (Slabbekoorn and Peet, 2003; Hanna *et al*., 2011; Luther and Derryberry, 2012). Therefore acoustic difference among populations, like differences in frequency (variation in both the minimum frequency of a song and dominant frequency of the trill), may be associated with ambient noise differences among sites rather than habitat structure (Mockford *et* al., 2011).

As observed in other tropical bird species (Pérez-Emán, 2005; Moore *et* al., 2005; Cadena and Cuervo, 2009; Gonzalez *et al*., 2011, Lougheed *et al*., 2013), Rufous-and-white Wrens showed high levels of genetic variation, even at regional scales. While some studies have suggested that acoustic patterns reflect vicariant events (González *et al*., 2011; Sosa-Lopez *et al*., 2014), our results indicate that acoustic variation does not reflect vicariant events, as observed in other studies (Wright and Wilkinson, 2001; Leader *et al.*, 2008; Ribot *et al*., 2012). This result is not surprising for males, given that mtDNA is maternally inherited and therefore reflects the movement of matrilineal lines historically. The mismatch between acoustic and mtDNA patterns for females further suggests the role that selection and drift have on acoustic patterns in Rufous-and-white Wrens.

Genetic analyses revealed that females disperse farther than males in Rufous-and-white Wrens. Increased dispersal capabilities and gene flow are suggested to increase acoustic similarity among sites (Lynch and Baker, 1994), and therefore we predicted that females would show less acoustic structure than males. Although pairwise comparisons of acoustic distance were greater for females than males, males and females showed similar patterns of acoustic structure. Given that females are more dispersive, our results seem most consistent with the idea that females learn their

songs following dispersal (Nelson *et al*., 2001; Wright *et al*., 2005). Learning songs post-dispersal, would maintain patterns of acoustic variation between populations, whereas learning pre-dispersal would homogenize acoustic variation (Lynch and Baker, 1994). Alternatively, different selection pressures and song learning preferences between sexes, and not dispersal differences, may drive patterns of acoustic variation between sexes (Mennill and Rogers, 2006). Further studies are necessary to quantify and compare patterns of song learning between sexes, and would aid studies of geographic variation in both male and female song.

Conclusion

We examined the relationship between acoustic divergence and ecological divergence and genetic divergence in male and female Rufous-and-white Wrens in Central America. Acoustic patterns were generally concordant between sexes, and our results indicate that dispersal is limited between populations and that cultural drift heavily influences acoustic variation. Acoustic distance was not correlated with ecological distance, although potential environmental differences among sites (including ambient noise or patterns of sound transmission) may influence acoustic divergence. Our results suggest that contemporary genetic patterns and not historical genetic patterns better reflect song variation in both male and female Rufous-and-white Wrens. Few studies to date have examined female song, and our study highlights the difficulties of studying female song; female Rufous-and-white Wrens sing fewer songs overall, less frequently, and more quietly than males, resulting in fewer recordings of high quality songs from females than males (500 vs 1600 male songs in our dataset, in spite of the same recording effort). Our study emphasizes the importance of studying female song, because studying male and female song patterns together may help to better understand the ecology and evolution of acoustic variation in tropical animals.

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Tables

Table 3.2: Percentage of male and female songs correctly assigned to the population they were recorded from using discriminant analysis. N equals the number of song typess analyzed from each population.

	Males		Females	
	N	**Population**	**N**	**Population**
Nicaragua	13	15.4%	-	-
Santa Rosa	32	43.8%	32	84.4%
Rincon	30	36.7%	19	47.4%
Monteverde	26	23.1%	18	11.1%
Central Valley	33	48.5%	10	40.0%
Overall	134	36.6%	79	53.2%

Table 3.3: Mean (± SE) values of male and female solo songs fine-structural measurements. Columns population, microsatellite and mtDNA represent the level used to distinguish songs using MANOVA. R^2 represents the total percentage of variance attributed to each variable within the three defined groups (values presented are the adjusted R^2 values). F reperesents the F-statistic for each variable and p represents the p value. Bold values indicate variables that were significant at p<0.05.

	Males				***Females***			
Measurement	***Mean ± SE***	***R^2***	***F***	***p***	***Mean ± SE***	***R^2***	***F***	***p***
Number of Syllables	14.63 ± 0.56	0.02	0.52	0.72	11.75 ± 0.53	0.05	1.41	0.25
Song Length (s)	2.21 ± 0.04	<0.01	1.12	0.35	1.89 ± 0.04	0.04	1.12	0.35
Intersyllable interval (s)	0.10 ± 0.01	0.06	1.90	0.11	0.11 ± 0.01	0.04	0.92	0.44
Length of Terminal Syllable (s)	0.15 ± 0.01	**0.29**	**12.96**	**<0.001**	0.14 ± 0.01	0.06	1.61	0.19
Dominant Frequency of the Trill (Hz)	893 ± 6	0.03	1.99	0.11	1065 ± 16	**0.11**	**2.98**	**0.04**
Bandwidth of Terminal Syllable (Hz)	342 ± 40	**0.09**	**3.07**	**0.02**	436 ± 58	**0.35**	**13.63**	**<0.001**
Maximum frequency of song (Hz)	1891 ± 40	0.02	0.57	0.62	2341 ± 61	0.02	0.46	0.71
Minimum Frequency of Song (Hz)	756 ± 7	**0.10**	**3.45**	**0.01**	934 ± 18	**0.19**	**5.68**	**0.001**

Table 3.4: Pairwise population comparisons of acoustic distances. All Acoustic distances represent Euclidean distances calculated from the population centroid using discriminant analysis. Values below diagonal areacoustic distances fro male songs; above the diagonal are acoustic distances for female songs.

	Nicaragua	Santa Rosa	Rincon	Monteverde	C. Valley
Nicaragua	-	NA	NA	NA	NA
Santa Rosa	1.26	-	2.30	1.59	2.41
Rincon	2.12	1.61	-	0.72	1.67
Monteverde	1.86	1.13	0.51	-	1.50
C. Valley	2.78	1.81	1.01	0.94	-

Table 3.5: Genetic diversity statistics for mtDNA and microsatellite results from five populations of Rufous-and-white Wren. N=Samples size; H_d=Haplotype diversity; π=nucleotide diversity; A_R=allelic richness; H_O=observed heterozygosity; H_E=expected heterozygosity; SE=standard error; F_{IS}=inbreeding coefficient.

	mtDNA				Microsatellites						
Population	**N**	**#haplotypes**	**H_d**	**π**	**N**	**A_R**	**H_O**	**SE**	**H_E**	**SE**	**F_{IS}**
Nicaragua	12	9	0.94	0.004	47	6.29	0.61	0.09	0.65	0.09	0.07
Santa Rosa	13	13	1.00	0.010	97	6.99	0.56	0.09	0.65	0.09	0.14
Rincon	10	9	0.98	0.010	30	6.37	0.56	0.09	0.64	0.10	0.13
Monteverde	13	8	0.91	0.003	27	6.47	0.62	0.08	0.69	0.08	0.10
Central Valley	9	5	0.72	0.002	10	6.06	0.60	0.09	0.67	0.09	0.11

Table 3.6: Genetic distance matrice of pairwise population comparisons; F_{ST} pairwise differences based on 10 microsatellite loci (below diagonal) and θ_{ST} pairwise differences based on ND2 mtDNA Gene (above diagonal). Bold values denote significant p-values following sequential Bonferroni corrections (adjusted p-value = 0.025 for θ_{ST})values. All F_{ST} values (microsatellite) were significant, p=0.0001.

	Nicaragua	Santa Rosa	Rincon	Monteverde	C. Valley
Nicaragua	-	**0.39**	**0.40**	**0.77**	**0.81**
Santa Rosa	**0.05**	-	-0.04	**0.29**	**0.31**
Rincon	**0.05**	**0.04**	-	**0.31**	**0.35**
Monteverde	**0.09**	**0.07**	**0.04**	-	0.02
C. Valley	**0.10**	**0.10**	**0.06**	**0.03**	-

Table 3.7: Results of causal modeling testing the relationship between acoustic distance and genetic distance, geographic distance and ecological distance for male and female Rufous-and-white Wrens. Italicized letters indicate the hypothesis tested from table 1 to determine the role of that variable on acoustic divergence. Test indicates the test conducted, Mantel and partial Mantle. *r* indicates the percent of variation explained for each model, while *p* indicates the associated p-value. All bolded values indicate the model is significant at p<0.05.

			Males		**Females**	
Dependent	**Variable**	**Test**	**r**	**p**	**r**	**p**
Acoustic adaptation						
Acoustic	ecological distance	Mantel	0.55	0.10	0.76	0.08
Acoustic	ecol|geog	partial Mantel	0.38	0.79	0.74	0.11
Acoustic	ecol|msat	partial Mantel	0.35	0.80	0.74	0.12
Acoustic	ecol|mtdna	partial Mantel	0.51	0.79	**0.88**	**0.04**
Dispersal						
Acoustic	geographic distance	Mantel	**0.79**	**0.02**	0.20	0.34
Acoustic	geog|ecol	partial Mantel	0.69	0.76	-0.29	0.87
Acoustic	geog|msat	partial Mantel	0.54	0.79	-0.51	0.19
Acoustic	geog|mtdna	partial Mantel	0.67	0.76	**0.72**	**0.03**
Genetic drift (microsatellites)						
Acoustic	msat genetic distance	Mantel	**0.67**	**0.04**	0.51	0.17
Acoustic	msat|geog	partial Mantel	0.19	0.83	0.66	0.81
Acoustic	msat|ecol	partial Mantel	0.52	0.77	-0.45	0.79
Acoustic	msat|mtdna	partial Mantel	0.42	0.79	**0.91**	**0.001**
Genetic Drift (mtDNA)						
Acoustic	mtDNA genetic distance	Mantel	0.70	0.06	-0.28	0.17
Acoustic	mtdna|geog	partial Mantel	-0.49	0.78	-0.73	0.78
Acoustic	mtdna|ecol	partial Mantel	0.60	0.77	-0.72	0.76
Acoustic	mtdna|msat	partial Mantel	0.40	0.79	**-0.88**	**0.01**

Figures

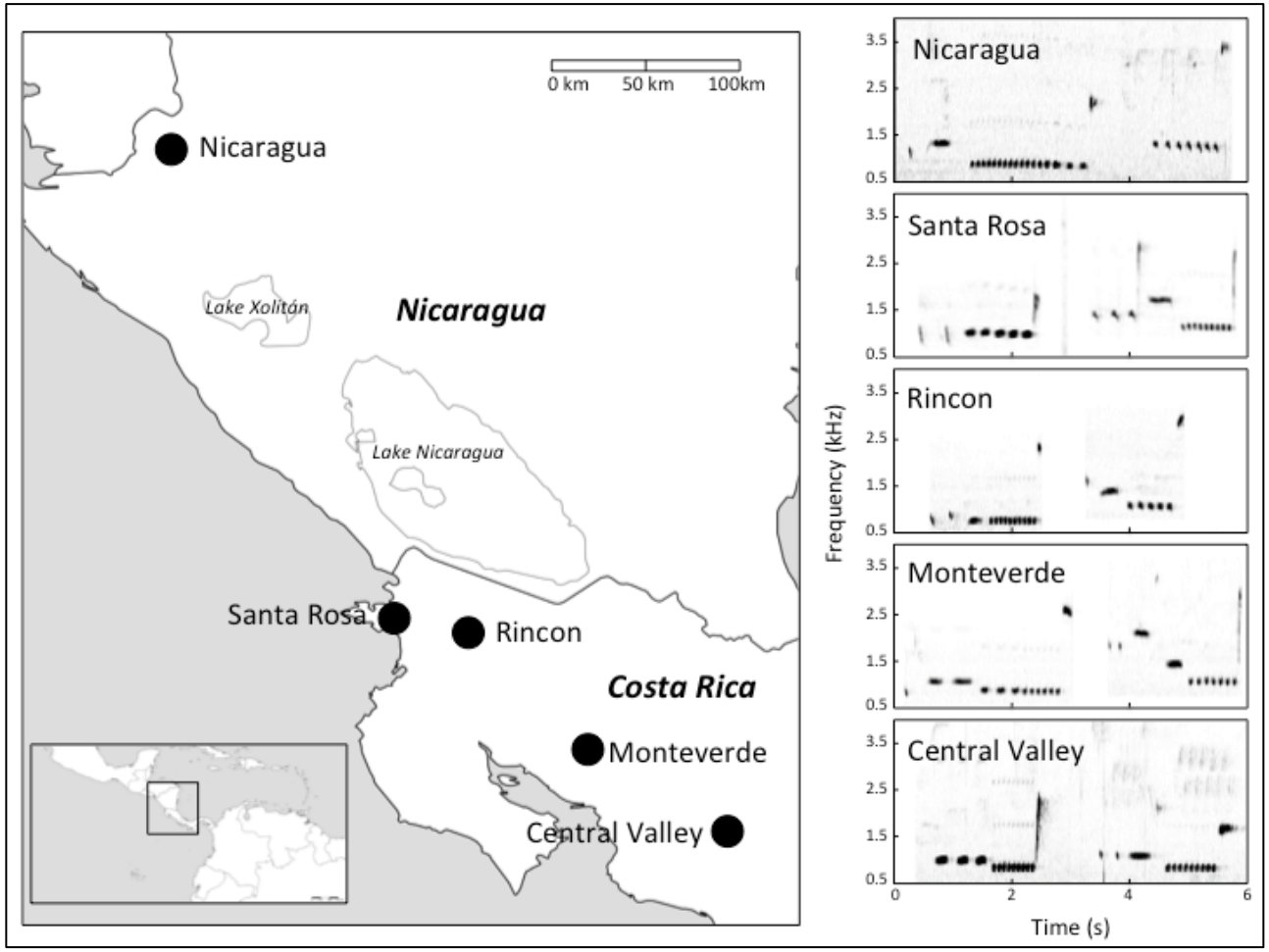

Figure 3.1: Map of the five populations of Rufous-and-white Wrens included in our analysis of acosutic and genetic variation. On the right are spectograms showing examples of male songs (on the left) and female songs (on the right) recorded from each of the five populations.

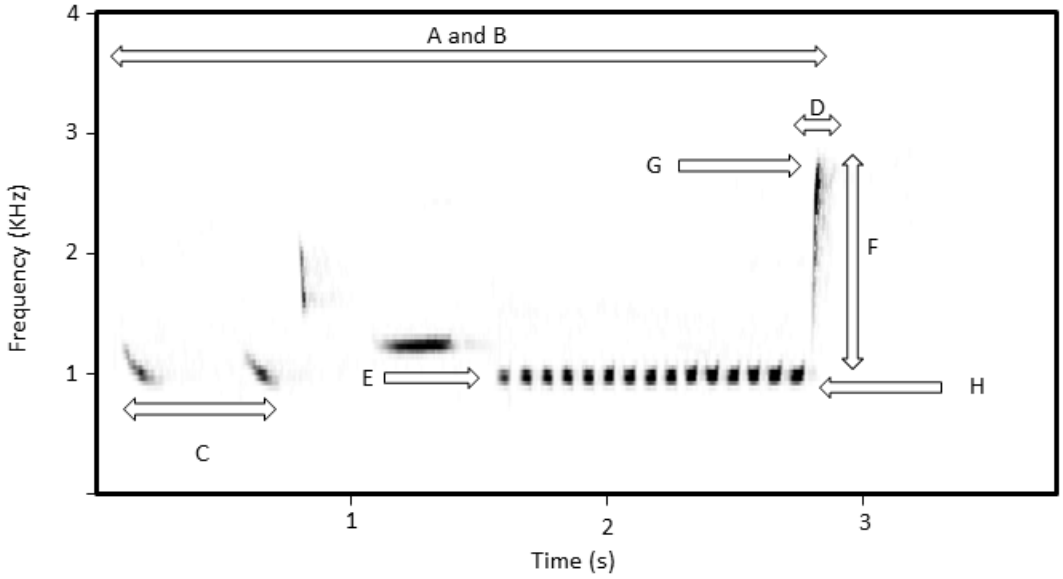

Figure 3.2: Spectogram of a male Rufous-and-white Wren song, showing the eight fine-structural measurements made for each male and female song. A=song length (s); B=number of syllables; C=inter-syllable interval (s; the average duration of silence between each syllable); D=length of terminal syllable (s); E=dominant frequency of the trill (Hz); F=bandwidth of the terminal syllable (Hz); G=maximum frequency of the song (Hz); H=minimum frequency of the song (Hz).

Supplementary information accompanying Chapter 3

Supplementary Methods

We used the Bayesian clustering model STRUCTURE version 2.3.3 (Pritchard *et al*., 2000) to investigate microsatellite population structure. For all runs we used the admixture model with the correlated allele frequencies model setting, but did not use sampling location as a prior. Each run consisted of a burn-in of 100,000 chains followed by 500,000 chains; we ran five iterations for each K, and K ranged from one to six (the maximum number of populations plus one). To determine the true K, we used the ΔK method (Evanno *et al*., 2005) as implemented in Structure Harvester (Earl and van Holdt, 2012). Following this initial run, we ran STRUCTURE (using the same settings) on the individual clusters containing more than one population to examine if there was hierarchical population structure within the recovered clusters.

To visualize the relationship among ND2 haplotypes and explore phyloegeographic structure, we constructed a statistical parsimony network for each gene using TCS version 1.21 (Clement *et* al., 2000).

Supplementary Results

Our analysis using STRUCTURE also revealed population structure among populations of Rufous-and-white Wrens (Figure 3.S1a). Using the ΔK method suggests that K=3 is the optimal K (ΔK =84.39); at K=3 STRUCTURE recognizes Santa Rosa and Nicaragua as separate clusters respectively, and recognizes Rincon, Monteverde, and Central Valley as a single cluster. Further analysis of the third cluster (i.e. Rincon, Monteverde and the Central Valley) using STRUCTURE revealed that K=2 was the optimal K (ΔK =0.48), separating Rincon

as a unique cluster from Monteverde and the Central Valley. In our initial analysis (K=3), 18 of 211 (8.5%) individuals were assigned to another cluster outside of their home cluster (Q>0.5). Five individuals showed evidence of introgression where the highest Q was to a cluster other than their home cluster but this values was <0.5. Taken together these results suggest potential migration among populations.

Our statistical parsimony network revealed two distinct haplogroups (Figure 3.S1b). These haplogroups separated geographically from North to South. In the North Nicaragua haplotypes were primarily distinct from Northwestern Costa Rica haplotypes. Within Northwestern Costa Rica (Santa Rosa and Rincon), however, we observed substantial admixture; 10 of the 23 individuals from Northwestern Costa Rica had Central Costa Rica (Monteverde and Central Valley) haplotypes.

Table 3.S1: Primer list and characteristics for the 10 microsatellite loci and 1 mtDNA gene used to genotype Rufous-and-white Wrens in this study; #alleles =total number of alleles for each locus, and T_A= Annealing temperature.

Locus	Sequence	Size	Repeat Motif	# alleles	T_A	Reference
Tru 08	F: ATCTTTGGGGTGAGTTAGGG	168-440	$(TAGA)_5$	35	50	New
	R: ACCTGTGGCACAATATTATCTATCT					
Tru11	F: ATGGCAGCAGACACCAGTTT	188-256	$(ATCC)_{12}$	18	50	New
	R: CAGGGAAGAAGATTGAGGATG					
Tru18	F: CCAGCCTCCAGCTACACAGT	156-244	$(GATG)_{20}$	21	50	New
	R: TTACTTCCCCAGTCCTGCTG					
Tru20	F: GAGAAGTATCCCATCCACATA	129-131	$(CA)_7$	2	50	New
	R: GAGAAGGGTCATCTTTGCCAGC					
Tru24	F: GCACCAGCTATTCCATCCAT	104-160	$(TCCA)_{18}$	14	54	New
	R: TTCCTGCTAAGGGCATCACT					
Tru25	F: GGAAGAGAGGGAGGAGGTGT	164-260	$(CTAT)_{11}$	17	50	New
	R: GGCACTGCTACACACAAACC					
RWWR-02c	F: CAAGTCTGCTTGTTAGAGCTGTCC	154-172	$(AGG)_8$	7	50	New
	R: GAAGTGCTGCTGGTGATGAG					
Th-Pl14	F: GTAAATTTCAGGAGTCCAGGTTGC	244-266	$(CA)_5$ (GACATACAGA) $(CA)_7$	12	58	Brar et al., 2007
	R: AAGCGCCCAAAATTAGCCAGAA					
Th-Pl26	F: TCAAATGTGCCACTGACTGAGT	176-180	$(GT)_8$	3	58	Brar et al., 2007
	R: AGCCTACTTCAAACTGAGACAGA					
Th-Pl30	F: ATGCCAGCACTAAAGAATGACAA	216-262	$(TG)_6$ TA $(TG)_3$	18	50	Brar et al., 2007
	R: CTACATAGCAGGCAGCAGAGGTT					
L5215	F: TATCGGGCCCATACCCCGAAAAT	1042	ND2 mtDNA gene		50	Hackett, 1996
H1064	R: CTTTGAAGGCCTTCGGTTTA					Drovetski et al., 2004

Table 3.S2: a) Environmental variable used to calculate ecological distance between populations. Lat=Latitude (N); Long=Longitude (W); Ppt. driest qrt.=precipitation during driest quarter (mm); Ppt coldest qrt.=precipitation during driest quarter (mm); Ann. Temp range=The annual range in temperature (annual maximum temperature-annual minimum temperature (°C); Temp. driest qrt.=the mean temperature during the driest quarter each year (°C); Env. noise=our categorical measurement of low frequency background noise at each population. We categorized sites as having low, medium, and high amounts of low frequency noise by visually inspecting spectrograms.

Population	**Lat (N)**	**Long (W)**	**Altitude (m)**	**Ppt. driest qrt. (mm)**	**Ppt. coldest qrt. (mm)**	**Ann. temp range (°C)**	**Temp driest Qrt. (°C)**	**Env. noise**	**Description of noise**
Nicaragua	13.27	-86.31	1206	76	144	14.2	19.7	1=Low	Low frequency background noise produced by wind and other biotic sources only
Santa Rosa	10.85	-85.60	286	14	519	14.3	24.8	1=Low	Low frequency background noise produced by wind and other biotic sources only
Rincon	10.78	-85.35	839	169	572	12.9	21.2	2=Med	Low frequency background noise produced by rivers
Monteverde	10.28	-84.80	1091	133	547	13.3	22.2	2= Med	Low frequency background noise produced by high winds; Average wind speed =24.6 km/h, with gusts up to 50 km
Central Valley	9.90	-84.25	831	48	530	13.1	23	3=High	Low frequency background noise produced by traffic noise and by small streams. Site is located in an a highly populated Urban area

Table 3.S3: Correlation coefficients from Discriminant Function Analyses loadings for each of the canonical variates using population to discriminate male and female songs

	Males				Females		
Measurements	**CV1**	**CV2**	**CV3**	**DF4**	**CV1**	**CV2**	**CV3**
Number of syllables	-0.139	**-0.423**	**-0.690**	**0.385**	-0.092	**0.365**	**0.407**
Song length (s)	0.217	**-0.569**	0.158	0.222	-0.006	**0.412**	0.122
Intersyllable interval (s)	0.177	**-0.331**	**0.321**	**0.368**	0.165	-0.137	-0.215
Length of terminal syllable (s)	0.174	**-0.471**	-0.083	-0.097	-0.174	**0.336**	0.233
Dominant frequency of the trill (Hz)	0.017	0.261	-0.137	-0.256	-0.256	**0.361**	**0.469**
Bandwidth of terminal syllable (Hz)	**-0.724**	-0.067	0.211	-0.242	**0.705**	**0.351**	0.021
Max Frequency of song (s)	0.018	-0.034	0.024	**0.714**	-0.020	0.231	0.235
Minimum frequency of song (Hz)	0.195	-0.170	0.103	0.013	**-0.362**	**0.596**	-0.104
Variation explained	69.9%	15.8%	11.1%	3.2%	75.3%	18.7%	6%

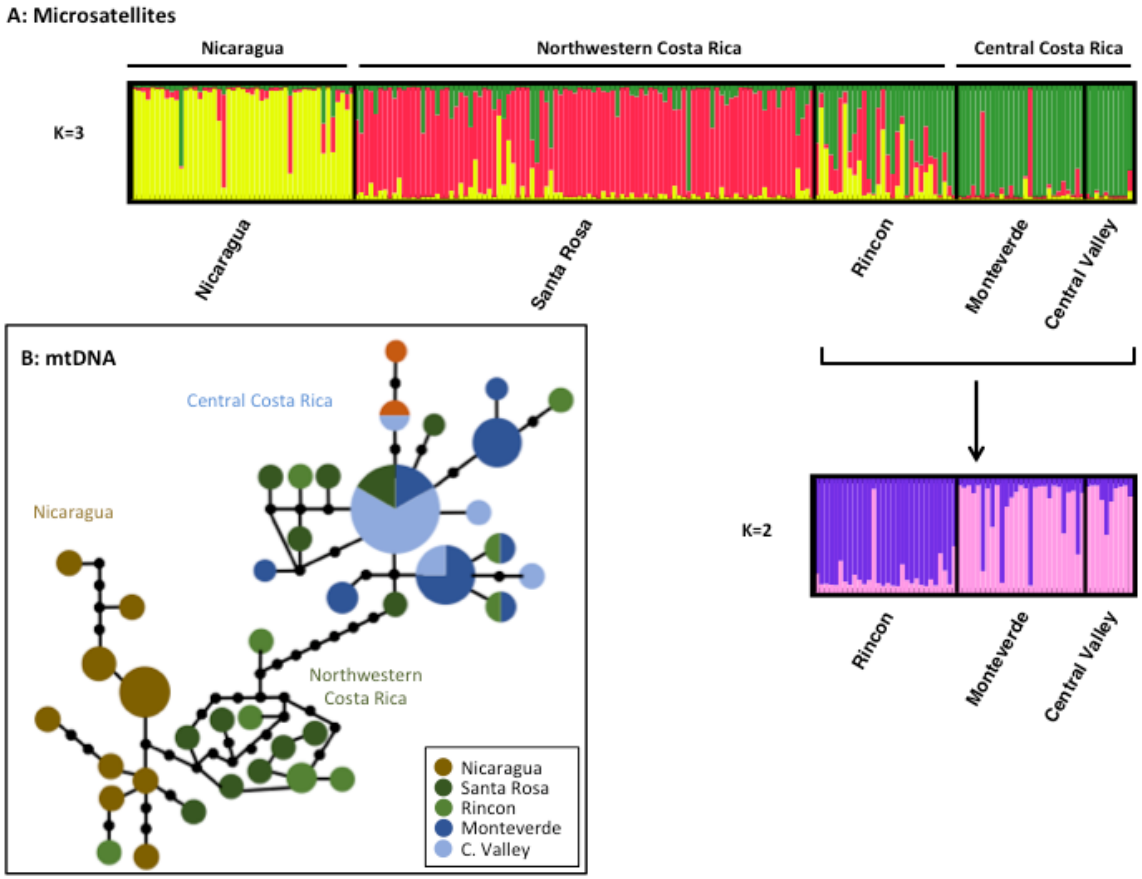

Figure 3.S1: (a) Genetic structure of five populations of Rufous-and-white Wrens along a 500 km transect. Summary of results of STRUCTURE using 211 individuals from five populations in Nicaragua and Costa Rica. Different colours coincide with group membership for the simulation at K=3. Fine-scale population structure analysis at K=2 indicates that Rincon is distinct from both Monteverde and Central Valley. (b) The statistical parsimony network showing the relationship between 57 individuals from five populations using a 1041 bp sequence of the ND2 gene. Coloured circles represent the number of individuals with the same haplotype, while the small black dots represent inferred/missing haplotypes. MtDNA groups are listed above the STRUCTURE histogram, as a reference for similarities and differences between microsatellite and mtDNA patterns

Chapter 4: Male and female first-generation migrants learn songs post-dispersal in a tropical bird where both sexes sing*

*This work is the outcome of joint research with D. Heath, R. Walter, and D. Mennill

Chapter Summary

A fundamental hypothesis about vocal learning is that young animals learn songs in their natal areas and, following post-natal dispersal, they introduce new songs into their breeding areas. We tested this hypothesis in a tropical bird, the Rufous-and-white Wren (*Thryophilus rufalbus*), where both sexes produce learned songs. We collected blood samples and acoustic recordings from 146 adult wrens from three populations in northwestern Costa Rica. We genotyped individuals at 10 polymorphic microsatellite loci and identified first-generation migrants using partial Bayesian genotype assignment. We quantified acoustic variation by comparing song sharing, repertoire novelty, and fine-scale acoustic structure between first-generation migrants and residents. We found significant population-level differences in acoustic structure of songs among the three populations. Of the 146 individuals genotyped, 9 individuals were identified as first-generation migrants. In contrast to our predictions, however, we found that these first-generation migrants did not exhibit differences in the acoustic structure of their songs from resident individuals in their breeding population, either for males or females. We conclude that first-generation migrants must learn local songs in their breeding populations, following post-natal dispersal. Acoustic differences between the three study sites imply the sustained divergent selective pressures at each site. Understanding and quantifying patterns of cultural evolution at multiple scales may help to explain why biodiversity is greater in the tropics and provide insight into how behavioural barriers, such as acoustic signals, contribute to population differentiation and even speciation.

Introduction

The magnitude, direction, and spatial scale of dispersal are all important components of gene flow, and contribute to genetic differentiation in natural populations (Bohonak, 1999). In addition to genetic divergence, dispersal influences phenotypic divergence, and in the absence of the gene flow that accompanies dispersal, phenotypic traits can diverge quickly (Lande, 1980; 1981; Irwin *et al*., 2001; Clegg and Phillimore, 2010). Examining variation in traits and behaviours in the context of dispersal patterns – especially those phenotypes associated with mate attraction and territory defense – can provide key insights into the relationship between phentoypic variation and gene flow (Wilkins *et al*., 2013).

The signals used by animals in social and sexual communication exhibit considerable geographic variation (reviewed in Bradbury and Vehrencamp, 2011). This has been especially well documented in acoustic signals (Prohl *et al*., 2006; Podos and Warren, 2007; Campbell *et al*., 2010). While most animals inherit their vocalizations innately, a few groups of animals have evolved vocal learning, including some birds, bats, primates, elephants, seals, and cetaceans (Janik and Slater, 1997; Jarvis, 2004; Poole *et al*., 2005; Sanvito *et al*., 2007). Vocal learning, whereby young animals learn to produce vocal signals after hearing sounds of conspecific animals, plays an important role in the cultural evolution of acoustic signals; copying errors (mutations), random cultural drift, selection, and dispersal can all give rise to new signals or change signal characteristics (Lynch, 1996). Vocal learning in birds occurs in three orders (Apodiformes, Psittaciformes, and Passeriformes) and provides a model system for studying the population-level implications of vocal learning. Most studies examining acoustic variation and learning, however, have focused on the vocalizations of males. Female birds also produce and learn songs, especially outside of north-temperate ecosystems, but little is known about song variation, development, and learning in females (Riebel *et al*, 2005), even though female song is an ancestral trait (Odom *et al*., 2014). Given that both males

and females sing in many tropical bird species (Slater *et al*., 2004), tropical birds offer an excellent system to examine patterns of song learning.

Examining songs in males and females in the context of dispersal is important, given that sex-biased dispersal is common in birds. Female birds regularly disperse greater distances than their corresponding males (Greenwood, 1980). As a result, life history differences between the sexes, including differences in dispersal behaviour, may influence acoustic variation and phenotypic evolution of males and females (Ortiz-Ramírez *et al*., 2016). If animals learn their vocalizations prior to dispersing from their natal populations, then they may introduce new signals into the populations to which they immigrate, and ultimately act to homogenize acoustic variation among populations (Wright *et al*., 2005). In contrast, if animals learn their vocalizations after dispersal, then this may promote acoustic divergence between populations (Ellers and Slabbekoorn, 2003).

In this study, we examine the role of immigration and acoustic variation in Rufous-and-white Wrens (*Thryophilus rufalbus*), year-round residents of Central America and northern South America. The singing behaviour of this species offers a unique system to explore song variation and song learning, given that both sexes sing and that individuals sing a variety of different songs (individuals can learn up to 15 songs; Mennill and Vehrencamp 2005; Harris et al. 2016). We combined molecular and acoustic analyses to answer the question: do first-generation migrants (i.e. birds born outside of their breeding population) exhibit acoustic differences compared to resident birds (i.e. birds born in their breeding populations)? We identified first-generation migrants in three Rufous-and-white Wren populations in northwestern Costa Rica using molecular genetic analyses. We then used acoustic analyses to compare the repertoires of males and females to determine if first-generation migrants have more novel songs in their vocal repertoires than resident birds. Finally, we compared the fine structure of the songs of residents to the songs of first-generation migrants, to see if the structure of the songs of first-generation migrants differs from the songs of residents.

Methods

In 2012 and 2013, we studied three populations of Rufous-and-white Wrens living at three sites in northwestern Costa Rica (Figure 4.1): Sector Santa Rosa of the Guanacaste Conservation Area (10.85 °N, 85.60 °W; hereafter "Santa Rosa"), Sector Rincon de la Vieja of the Guanacaste Conservation Area (10.78 °N, 85.35 °W; hereafter "Rincon"), and University of Georgia Campus in the San Luis Valley near Monteverde (10.28 °N, 84.79 °W; hereafter "Monteverde"). We captured birds at each population using mist nets, and banded each bird with a unique band combination that included three colour bands and one numbered aluminum band. From each bird we collected a small blood sample (~100 μl) from the brachial vein, and stored blood samples in 95% ethanol or Queen's Lysis Buffer (Seutin *et al*., 1991). Individuals were sexed based on the presence of a brood patch (females) and by singing behaviour (sexes can be distinguished based on fine structural differences in songs; Mennill and Vehrencamp 2005). Additionally, we collected samples from birds in two other populations: we included one population north of our three focal populations in northwestern Nicaragua (13.27 °N, 86.31 °W) and one population to the south of our three focal populations in the Central Valley of Costa Rica (9.90 °N, 84.25 °W). These two additional populations were included in our analysis to improve our ability to detect potential first-generation migrants at our three focal populations, but these individuals were not included in the acoustic analyses in the current study, or the combined acoustic and genetic analyses because we did not have a sufficient acoustic recordings for these two populations.

Genetic analysis

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega), and individuals were genotyped at 10 microsatellite loci. We used four previously designed microsatellite primer sets: *Th-Pl 14, Th-Pl 20, Th-Pl 30* (Brar *et al*., 2007), *RWWR 2c* (H. Mays, personal communication). In addition, we developed six new microsatellite primer sets following the

microsatellite enrichment procedure detailed in Walter et al. (2007): *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24,* and *Tru 25*. All PCR reactions were conducted in 12.5 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 1.25 μL of 10x PCR buffer (Applied Biosystems), 0.5 μL of $MgCl_2$ (2.5 mM), 0.45 μl of dNTPs (0.2 mM), 0.05 μl of bovine serum albumin, and 0.5 U of Taq (Genscript, Applied Biosystems). For the primer sets *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25*, and *RWWR 2c,* we included 1 μM each of the tel-forward, reverse, and M13 dye-labeled primer (GTAAAACGACGGCCAGT). For the remaining three primer sets (*Th-Pl 14, Th-Pl 20,* and *Th-Pl 30*) we used 1 μM each of the forward primer and the IR-dye labeled reverse primer. PCR conditions for *Th-Pl 14, Th-Pl 20,* and *Th-Pl 30* followed those outlined in Douglas *et al*., (2012), while for the remaining primer sets we used the following PCR conditions: one cycle of 94.0 °C for 2 minutes, followed by 34 cycles of 94.0 °C for 10 seconds, 50.0 °C for 10 seconds, 72 °C for 30 seconds, followed by a final extension cycle of 72 °C for 90 seconds, although for the primer set *Tru 24* we increased the annealing temperature (T_2) to 54 °C to eliminate stuttering. PCR products were visualized on a 6% acrylamide gel on a Licor 4300 DNA analyzer (version 1.3.8-1, Biosciences). To ensure consistent sizing and scoring across gels, we ran controls with known size standards on each run. Finally allele sizes were scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

We genotyped 211 Rufous-and-white Wrens from five populations. The majority of loci × population comparisons did not show significant departures from HWE (only three of fifty loci × population comparisons, 6%, departed from HWE) or linkage disequilibrium (1 of 225 comparisons, 0.004%, showed evidence of linkage disequilibrium) following sequential Bonferroni comparisons. Two of the three loci × population combinations that showed departures from HWE were found at Santa Rosa; to further ensure that departures from HWE were not driving the observed patterns, we performed our analysis with all 10 loci and then repeated the analyses without the two loci that

showed significant departures from HWE (*ThPl-14* and *ThPl-30*). Removing these loci did not change our results and therefore we present results including all 10 loci.

To identify first-generation migrants, we used the "detect migrants" function implemented in the program GENECLASS 2.0 (Piry *et al*., 2004). This analysis detects first-generation migrants (i.e. birds born outside of the sampled population), using likelihood ratio statistics and Monte-Carlo resampling methods. We identified first-generation migrants using the likelihood ratio of L_{home} and Bayesian resampling method derived by Paetkau *et al*. (2004) and criteria suggested by Rannala and Mountain (1997). While the L_{home} to L_{max} likelihood ratio method has greater statistical power than any of the other methods implemented in the program (Paetkau *et* al., 2004; Piry *et al*., 2004), we also used the L_{Home} likelihood ratio because this method is recommended by Paetkau *et al*. (2004) suggests it is not the most appropriate when all potential source populations of first-generation migrants haven't been sampled. Our resampling method simulated 10,000 individuals, and we identified individuals as first-generation migrants if the probability of them being excluded from the population they were banded at was less than 0.05.

We complemented our migrant detection analysis with Bayesian exclusion analysis (Rannalla and Mountain, 1997) in GENECLASS 2.0. The purpose of this analysis was to further characterize dispersal patterns in this species, and to test the accuracy of our first-generation migrant analysis, following Sunnucks (2011). Comparing results from these complementary analyses will help to avoid potential type I errors, given that the exclusion analysis tests the probability of an individual originating from another population (Sunnucks, 2011; García-Navas *et al*., 2014). For our exclusion analysis, we used the same resampling method that we used for first-generation migrant analysis. Individuals were excluded from the population where they were banded if the probability was less than 0.05 and assigned to another population based on their likelihood values.

Analyses of song repertoires

We recorded birds during the breeding season, in April through July of each year of the study, when vocal output is high for this species (Topp and Mennill, 2007). Overall, we recorded songs from 146 birds (89 males and 57 females) from our three study populations. From these three populations, we identified 51 male and 44 female song types at Santa Rosa, 36 male and 24 female song types at Rincon, and 33 male and 20 female song types at Monteverde. We recorded each individual on at least two separate occasions (average = 5.63 ± 0.33, range 2-12). The majority of our recordings were collected during focal recordings, where we followed each bird around throughout its territory (each morning, from 0445h to 1100h) and confirmed the bird's identity during the recording. Songs were collected during focal recordings using a solid-state digital recorder (PMD-660 Marantz; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70). We supplemented these recordings with recordings from automated digital recorders (model: Song Meter 2, Wildlife Acoustics Inc., Concord, Massachusetts, USA; Mennill *et al*., 2012). We placed these recorders within the center of the territories of each focal pair, usually within 10m of the pair's nest. We confirmed that the songs collected by these automated recorders were those of the intended pair, by re-sighting the focal individuals in their territory after each automated recording session, and by matching the songs collected during focal recordings (as in Harris *et al.*, 2016).

We used several methods to quantify repertoire variation in Rufous-and-white Wrens. First we calculated repertoire size for each individual, as in previous studies of this species (Mennill and Vehrencamp 2005; Harris et al. 2016), calculating the number of song types each individual sang. To do this, we annotated all audio files using SYRINX-PC sound analysis software (J. Burt, Seattle, Washington, USA), and for each male and female we built a song library of all the songs in the repertoire of each bird using simple enumeration (*sensu* Harris *et al*., 2016). Next we calculated the

percentage of songs that an individual shared with all other individuals of the same sex within the population they were recorded at. To classify song types, we inspected the fine-structural characteristics of songs following the approach outlined in Harris *et al*. (2016). Previous work by Barker (2008) has shown that discriminant analysis can differentiate song types based on fine-structural measurements (i.e. duration of song, maximum frequency, minimum frequency, and inter-syllable interval), and we incorporated these methods to help assign song types correctly. We defined song sharing as the proportion of songs shared between two individuals. To measure song sharing, we calculated an adjusted Jaccard's coefficient (S_j) of sharing using the following formula (Tracey and Baker 1999) in R 3.2.3 (R Core Team, 2014):

$$S_j = c/((a+b+c)-d)$$

where a = the number of song types in individual A's repertoire but not individual B's, b = the number of song types in individual B's repertoire but not individual A's, c = the number of song types shared between two individuals, and d = the difference in repertoire size between individual A and B. We chose this coefficient, because this method accounts for differences in repertoire size (d) providing a more accurate estimate of sharing between two individuals. In our analysis of repertoire sharing we present the average percentage of songs that an individual shares with all members of the same sex in their population.

We considered a song to be shared between two individuals if they met the following criteria: (i) songs shared the same sequence of elements in the introductory part of the song (although we ignored differences of one or two syllables in this section because first notes are produced quietly and sometimes difficult to detect); (ii) introductory syllables were produced at the same frequency (within100 Hz); (iii) trills were composed of the same type of syllables (i.e syllables were the same length and shape); (iv) trills were produced at the same frequency (within 100 Hz); (v) trills were delivered at the same rate (within two elements / s); (vi) terminal syllables were the

same shape (e.g. long tonal syllables that covered a short bandwidth versus short syllables with a broad bandwidth).

To quantify how unique an individual's repertoire was, relative to the rest of the population where it was recorded, we calculated a measure we call "repertoire novelty". Following Fayet *et al*. (2014), each song type was given a weighted value based on how common it was in the population (i.e. the number of individuals with this song type in their repertoire); common song types received a low value (e.g. a song present in 80% or more of the individuals in the population received a value of 1), while rare song types received a higher value (e.g. a song sung by a single individual received a value of 6; see supplementary methods for details). We then added the accumulated values for every song in each bird's repertoire and divided this sum by the repertoire size of the individual; this gave us an estimate of how novel the bird's repertoire was. For example, an individual with a repertoire that included 10 widespread songs would have a novelty score of 1, while an individual who sang 10 songs that were not shared with any other bird in the population would receive a novelty score of 6.

Although most of the birds we recorded were banded, to accurately estimate our within-population song sharing and song repertoire novelty analyses, we included several unbanded birds that we recorded at Rincon and Monteverde. We included one unbanded male and eight unbanded females from Monteverde, and one unbanded male and five unbanded females from Rincon in our analysis. We were able to recognize these unbanded individuals based on their location (the territory they occupied), their association with the mate (all unbanded individuals were paired with a banded individual), and the consistency of their repertoire (we compared repertoires between recording sessions, to ensure that we were recording the same bird on each occasion).

Analyses of song structure

To compare the fine-structural details of the songs of resident birds and first-generation migrants, we selected and measured a subset of each bird's vocal repertoire (Figure 4.2 and Figure 4.3). For males we selected and measured four song types from each population (n=12). We chose twelve song types that represented the most common song types in each population, and we measured these song types because they were found in the majority of the repertoires of each male we analyzed. For females we followed a similar approach, at first by targeting the four most common song types in each population; but given that females sing less often and fewer songs overall, we had to expand our selection criteria and measured up to 16 different song types per population (n=44 across the three populations). To measure the fine-structural differences in songs, we only included songs with a high signal-to-noise ratio.

In this anlayses we measured 859 songs from 85 males and 476 songs from 54 females (4 males and 4 females were excluded due to high background noise in recordings, which made fine structural analysis difficult). Whenever possible, we tried to include songs from multiple recordings (i.e. from different days, to eliminate any bias from recording on a single day) and measured up to three exemplars per song type per recording. We measured up to six exemplars of each song type for each bird (males: average number of songs measured=10.21 songs, range 1-21; average number of song types measured for each male was 3.26 song types, range 1-4; females: average number of songs measured=8.81 songs, range 1-32; average number of song types measured for each female was 4.16 song types, range 1-9).

To quantify fine-structural variation in the songs of male and female Rufous-and-white Wrens we collected five temporal and spectral measurements of their songs. For each song we measured the duration of the song (s), element rate of the trill (the number of elements/second in the trill portion of the song), dominant frequency of the trill (Hz), minimum frequency of the song

(Hz), and maximum frequency of the song (Hz). We used the automated parameter measurements tool in AviSoft-SASLab Pro (version: 5.2.04; R. Sprecht; Berlin, Germany) to measure these features, thereby minimizing subjectivity in the fine-structural measurements. Songs were resampled to 8000 Hz, which allowed maximum spectral resolution. For each song we created a spectrogram, with an effective resolution of 8 Hz and 4 ms (settings: transform size: 1024 Hz; overlap: 96.86%; window: Hamming). We used a high pass filter of 500 Hz to remove any low-frequency background noise from the sound files. All measurements used for all statistical analysis represent means for each individual bird. To account for differences in sampling, we calculated an average across all the songs that we measured for each individual; we then used the average of these measurements for each of the five variables in our subsequent analyses of differences in the fine scale structure of songs.

Statistical analyses

We compared repertoire size using an analysis of variance (ANOVA), with repertoire size as our response variable and migrant status (i.e. residents vs. first-generation migrant) and population (i.e. the population where we sampled the bird) as our independent variables. We ran a separate model for each sex. Next we tested for differences in repertoire composition (i.e. within-population song sharing and repertoire novelty) between first-generation migrants and residents using linear mixed models. Again we ran a separate model for each sex, using within-population song sharing and repertoire novelty as our response variable and migrant status and population as our independent variables; we also included repertoire size as a covariate, to account for repertoire size differences among individuals. Finally, to test for differences in fine structure of songs, we performed a separate ANOVA for each sex, using the five fine-structural measurements as our response variables, and migrant status and population as the independent variables in our model. We tested all variables for normality by viewing Q-plots of the residuals and all values are presented

as the standard mean ± the standard error. All statistical analyses were carried out in SPSS (version 23.0, SPSS Inc., Chicago, IL, USA).

Results

Genetic analyses

We detected 16 first-generation migrants using GENECLASS (based on all 211 birds in our dataset; Table 4.1). Fourteen of the sixteen individuals (88%) identified as first-generation migrants were also identified as mismatches in our complimentary GENECLASS population exclusion analysis, demonstrating that the same individuals were identified as first-generation migrants using two different approaches. The number of first-generation migrants in each population ranged from two to four individuals. Overall 6.3% (8 of 127) of the males we sampled were identified first-generation migrants, while 9.5% (8 of 84) of the females we genotyped were identified as first-generation migrants; we found no significant difference, however, in the number of males and females identified as first-generation migrants (binomial test, $p=1.0$).

Song analyses

Based on our acoustic recordings of the males and females from three populations, we identified a number of unique song types that only a single individual sang in each population (15 male and 9 female song types from Santa Rosa; 11 male and 6 female song types from Rincon; and 4 male and 5 female song types from Monteverde were unique; Table 4.2). Of the 29 male and 20 female unique song types we recorded, male and female first-generation migrants sang significantly fewer unique song types than residents (binomial test, males: 14%, 4 of 29 $p=0.001$; females: 5%, 1 of 20, $p<0.001$). Furthermore, we plotted the frequency of unique song types with genetic assignment index (Figure 4.4), to examine whether unique song types were produced by birds with non-local genotypes (i.e. negative assignment indices). We found no significant difference for both males and females (binomial test, males: $p=0.35$; females: $p=1.0$), and individuals with non-local

genotypes accounted for 40% (12 of 30) and 53% (10 of 19) of the male and female unique songs we recorded in the three populations.

Repertoire size comparisons show that the repertoire size of neither male nor female first-generation migrants (males: 11.55±0.27 song types; females: 6.67±0.39 song types; Table 4.2) was significantly different from resident males (12.50±0.89 song types; $F_{1, 84}$= 1.04, p=0.31) and females (6.67±1.19 song types; $F_{1, 51}$= 0.00, p= 0.99). When we conducted this analysis for each of population separately, repertoire size was not significantly different between both male and female first-generation migrants and residents (Table 4.2). We did, however, observe song repertoire size differences among populations. Whereas males showed a significant difference (mean = 11.93±0.39 song types; $F_{2, 84}$=5.20, p=0.01), females showed no significant difference in song repertoire size among populations (mean= 6.67 ± 0.63 song types; $F_{2, 51}$= 0.26, p=0.77); post-hoc comparisons revealed that males from Rincon (13.15±0.74 song types) had significantly larger (p=0.01) repertoires than males from Santa Rosa (10.75±0.25 song types), while males from Monteverde (11.29±0.63 song types) showed no significant difference in repertoire size from males in the other two populations (p>0.18).

Overall, male and female first-generation migrants showed similar rates of song-sharing with residents (males: 0.47±0.01 for first-generation migrants versus 0.47±0.03 for residents; $F_{1,82}$=0.00, p=0.99; females: 0.40±0.05 for first-generation migrants versus 0.41±0.02 for residents; $F_{1,50}$= 0.08, p=0.78; Table 4.2). Similarly, we found no population-level differences between first-generation migrants and residents when we analyzed each of the three populations separately for either sex (p>0.17; Table 4.2). Although, we did not observe significant differences between populations in within-population song sharing for males ($F_{2,83}$=2.55, p<0.08; Table 4.S1), we did observe significant differences for females ($F_{2,50}$=20.12, p<0.001); females from Monteverde showed significantly higher rates of song sharing (0.63±0.05; p<0.001) than females at Rincon (0.33±0.05)

and Santa Rosa (0.25±0.03). Song sharing was not significantly correlated with repertoire size for either sex (males: F=3.36, p=0.04, partial eta^2=0.06; Females: F=3.02, p=0.09, partial eta^2=0.06).

Repertoire novelty scores (i.e. a measure of how unique the songs are within an animal's repertoire compared to others in the population) were comparable between male first-generation migrants (2.63±0.14) and residents (2.53±0.04; $F_{1,83}$=0.55, p=0.46; Table 4.2), and between female first-generation migrants (2.99±0.26) and residents (3.21±0.08; $F_{1,50}$=0.64, p=0.34). For males, we observed borderline non-significant population-level differences in repertoire novelty scores ($F_{2,83}$=2.86, p=0.06); however, repertoire novelty scores were significantly different among populations for females ($F_{2,50}$=12.51, p<0.001). Females from Santa Rosa (3.92±0.18) had significantly higher repertoire novelty scores (p<0.01) than females from Rincon (2.95±0.26) and Monteverde (2.44 ± 0.26). Repertoire novelty was significantly correlated with repertoire size, and individuals with larger repertoires had more novel repertoires than individuals with smaller repertoires (males: F=8.31, p=0.005, partial eta^2=0.09; females: 6.06, p=0.02, partial eta^2=0.06=0.11).

Overall the fine structure of the songs of male and female first-generation migrants was similar to that of resident birds. Analysis of variance revealed no differences in the structure of songs shared by both first-generation migrants and residents (Table 4.3). Although we did not observe differences between first-generation migrants and residents, we did observe population-level differences in the fine structure of male and female songs (Table 4.S2). When comparing the fine structure across the three populations, males showed differences in the fine structure of songs for all five variables that we measured (duration of song, element rate of the trill, dominant frequency of the trill, and the maximum and minimum frequency of the song; Table 4.S2), while females showed structural differences for two of the five variables we measured (duration of the song and minimum frequency of the song).

Discussion

Dispersal plays an important role in the evolution of animals' acoustic signals (Lynch, 1996) and given the important role that acoustic signals play in resource competition and mate choice (Bradbury and Vehrencamp, 2011), the interplay between dispersal and signal divergence may shape patterns of acoustic evolution. We studied three features of the songs of Rufous-and-white Wrens in three populations in Costa Rica: within-population song sharing, repertoire novelty, and acoustic structure of male and female song. We compared these acoustic features between birds classified as first-generation migrants or residents using genetic analyses. Although previous work has suggested that immigration and dispersal increases song diversity and influences acoustic structure (Stewart and MacDougall-Shackleton, 2008; Fayet *et al*., 2014), we did not observe this in our study. First-generation migrants and residents showed no differences in within-population song sharing, repertoire novelty, or fine-scale acoustic structure, and these patterns were consistent for both males and females. Overall our results imply that male and female Rufous-and-white Wrens learn songs post-dispersal in their breeding populations, as opposed to learning songs in natal populations. Although we did not observe any differences in the songs and repertoires of residents versus first-generation migrants, we did observe differences in singing behaviour among populations, overall the three study populations showed acoustic differences that were present in the songs of both male and female birds. Below we discuss our findings and the factors that may influence the limited role that immigration appears to play in cultural diversity in our study system.

Dispersal and acoustic variation

Genetic assignment methods revealed gene flow among five populations of Rufous-and-white Wrens in Central America (Chapter 3). Eight percent of the individuals we genotyped were first-generation migrants, which is comparable to migrant rates observed in other resident bird species (Moore *et* al., 2005; Pruett and Winker, 2005; García-Navas *et al.,* 2014). By comparison,

these rates are much lower than those observed in two non-passerine species studied in the same region of Central America (McDonald, 2003; Wright *et* al., 2005); these studies showed that parrots and manakins move among populations, resulting in little genetic differentiation between populations. Differences in genetic patterns between wrens, parrots, and manakins, suggest that differences in dispersal capabilities among the three species may explain differences in genetic divergence (Claramunt *et al*., 2012). In particular, parrots and manakins are known to have high dispersal capabilities (McDonald, 2001; Wright *et al.*, 2005), whereas insectivorous understory birds like wrens are thought to have lower dispersal capabilities (Stouffer and Bierregaard 1995; Şekercioğlu *et al*., 2002; Moore *et al.*, 2008).

To date, most studies of dispersal and acoustic divergence have focused on temperate animals (Stewart and MacDougall-Shackleton, 2008; Fayet *et al*., 2014; but see Wright *et al*., 2005). Tropical animals exhibit stronger patterns of philopatry than temperate animals (Stutchbury and Morton, 2008) and these life history differences between temperate and tropical animals may explain why we saw no acoustic differences between residents and first-generation migrants in our study. Furthermore, many studies, including previous work on Rufous-and-white Wrens (Chapter 3), have demonstrated that genetic and acoustic variation are not correlated (Wright and Wilkinson, 2001; Ruegg *et al.*, 2006; Ortiz-Ramírez *et al.,* 2016; but see MacDougall-Shackleton and MacDougall-Shackleton, 2001). Therefore, the results in our study may arise from the lack of a correlation between acoustic and genetic variation. While acoustic and genetic traits often show similar patterns of variation (Ruegg *et al.*, 2006; Ortiz-Ramírez *et al.,* 2016), this is usually due to other factors like drift and selection in the presence of isolation acting on both cultural and biological evolution simultaneously. This pattern may be even more prominent in tropical animals, given that dispersal and ecological specialization are considered to be strong drivers of speciation in the tropics (Claramunt *et al*., 2012; Salisbury *et al*., 2012; Smith *et al*., 2014).

Song learning

Although we found no differences between the singing behaviour and acoustic structure of the songs of first-generation migrants versus residents, our results provide insight into dispersal and song learning behaviour in Rufous-and-white Wrens. First, given that Rufous-and-white Wren first-generation migrants possessed very few unique songs in their repertoires, our results suggest that they do not introduce new songs into the populations that they disperse into, contrary to the pattern observed in other systems where immigrant birds provide an influx of novel acoustic information (e.g. Payne, 1996; Wright *et al.*, 2005; Gammons and Baker, 2006; Stewart and MacDougall-Shackleton, 2008). This observation provides insight into the role of dispersal on cultural patterns, and also the timing of dispersal in this species. Rufous-and-white Wrens are closed-ended learners; in more than a decade of study we have no evidence of a bird incorporating a new song type after their first year (Harris *et al*., 2016). This suggests that dispersal events must occur during the first year, when these animals are still learning their songs, and that young birds learn songs after dispersal. If birds were learning songs prior to dispersal, or if they disperse after their first year, then we would expect to see birds with repertoires and vocal properties different from resident birds (Salinas-Melgoza and Wright, 2012).

Our three study populations shared relatively few song types, but we did observe a number of population-specific song types that were common to the repertoires of males and females in each population. The prevalence of local song types may occur due to male and female song type preferences that develop during the song-learning period (Grant and Grant, 1996). Additionally, females and males may select mates based on their ability to produce local song types (Nowicki *et al*., 1998; Reinhold, 2004). Many birds have been shown to respond more strongly to local songs or local dialects (Danner *et al*., 2011; Dingle *et al*., 2009; 2010; Derryberry *et al*., 2011; Garamszegi *et al*., 2012; Caro *et al*., 2013), including Rufous-and-white Wrens (Hick *et al*., 2015). Several of these

studies have suggested that the decreased response to non-local songs may be indicative of song acting as a reproductive barrier (Irwin *et al*., 2001). Alternatively, the reduced response may be due to these songs containing less information than local songs, given that different song types are known to be used in different contexts by some species (Trillo and Vehrencamp, 2005; Cardoso *et al*., 2009; Demko *et al*., 2013), including the Banded Wren (*Thryophilus pleurostictus*), a closely related congener of the Rufous-and-white Wren.

While we did not observe any differences between the songs and song repertoires of residents and first-generation migrants, we did observe considerable variation among our three study populations. In particular we noticed significant differences in repertoire size for males, and within-population song sharing for both males and females. Whether between-population behavioural differences are influenced by genetic factors, developmental factors (Nowicki *et al*., 1998; Reinhold, 2004), social factors (Williams and Slater, 1990), or a combination of the three, requires further examination.

Among our three study sites we observed differences in the territory sizes of individuals: Rufous-and-white Wrens at Monteverde and Rincon occupy much smaller territories than males at Santa Rosa (60 m^2 versus 100m^2 respectively). The former populations have higher densities and therefore males in these populations may learn more songs because they have more neighbours nearby, creating more opportunity for hearing tutor songs. While we observed no significant differences in within-population song sharing for males, we observed significant differences for females. Female repertoires at Monteverde had much higher sharing levels than females at Santa Rosa; this could reflect the higher population density at Monteverde. Demographic factors may play a role in song sharing, but further studies are necessary to see how factors like proximity of neighbours and population density influence song sharing, repertoire size, and aspects of vocal behaviour (Williams and Slater, 1990).

Conclusion

We studied the influence that first-generation migrants have on the cultural diversity of male and female Rufous-and-white Wrens. Contrary to other studies (Stewart and MacDougall-Shackleton, 2008; Fayet *et al*., 2014), we did not find that first-generation migrants introduce unique songs into their breeding territories or that they differ in song structure from that of residents. Our results suggest that annual dispersal among populations is relatively low in this species, and this likely reflects the strong philopatric nature of this species. Importantly, our study suggests that first-generation migrants learn songs from their breeding population and reproduce these songs similar to resident males and females. Additionally, our results suggest that dispersal events in this species must be restricted to the first year when these animals are still learning their songs. Furthermore, the prevalence of local song types may reflect selection for specific songs within each population. Further studies are necessary to better understand why these songs are continually learned in each population, and to determine why these animals possess song repertoires, and how they use their songs. Additional studies will also help to provide greater insight into female song, duetting, and the evolution and function of these signals.

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Tables

Table 4.1: Number of Rufous-and-white Wrens detected as first-generation migrants, and the number of individuals assigned to another population based on exclusion analysis at each population. The two methods showed high concordance, and we found no significant difference between sexes with respect to the number of males and females identified as first-generation migrants or assigned to an alternative population.

	First-generation Migrant Analysis		Population Exclusion Analysis				
Population	**Residents**	**First-generation Migrants**	**Nicaragua**	**Santa Rosa**	**Rincón**	**Monteverde**	**Central Valley**
Nicaragua	44	3	45	1	0	0	1
Santa Rosa	95	2	0	96	0	1	0
Rincón	26	4	0	2	26	2	0
Monteverde	23	4	0	1	2	23	1
Central Valley	7	3	0	0	1	2	7

Table 4.2: Differences (mean ± standard error) in the vocal repertoires of resident and first-generation migrant Rufous-and-white Wren males and females. The number of unique songs represents the total number of songs attributed to each group. Statistics presented are for each population and overall from ANOVA (repertoire size) and ANCOVA (song sharing and repertoire novelty) linear models. Male and female first-generation migrants and residents showed no significant differences in their vocal repertoires (p>0.05).

		Females				Males			
Population	**Measurement**	**Resident**	**First-generation migrant**	**Test**	**p**	**Resident**	**First-generation migrant**	**Test**	**p**
Santa Rosa	Repertoire Size	7.22±0.38	7.00±1.60	$F_{1,51}$ =0.02	0.89	10.74±0.25	-	-	-
	Song Sharing	0.25±0.02	0.25±0.07	$F_{1,50}$ =0.01	0.92	0.47±0.01	-	-	-
	Repertoire Novelty	3.94±0.08	3.90±0.34	$F_{1,50}$ =0.02	0.90	2.58±0.04	-	-	-
	No. of Unique Songs	9	0			15	0		
	Sample Size	37	2			59	0		
Rincon	Repertoire Size	6.67±0.75	7.00±2.26	$F_{1,51}$=0.02	0.89	12.31±0.54	14.00±1.38	$F_{1,84}$ =1.30	0.26
	Song Sharing	0.41±0.03	0.26±0.09	$F_{1,50}$ =2.17	0.15	0.52±0.02	0.49±0.04	$F_{1,83}$ =0.25	0.62
	Repertoire Novelty	3.05±0.15	2.85±0.49	$F_{1,50}$ =0.14	0.71	2.31±0.08	2.47±0.21	$F_{1,83}$ =0.51	0.48
	No. of Unique Songs	5	1			8	3		
	Sample Size	9	1			13	2		
Monteverde	Repertoire Size	6.13 ± 0.80	6.00±2.26	$F_{1,51}$ =0.00	0.96	11.58±0.56	11.00±1.13	$F_{1,84}$ =0.21	0.65
	Song Sharing	0.58 ± 0.03	0.67±0.09	$F_{1,51}$ =0.90	0.35	0.41±0.02	0.44±0.04	$F_{1,83}$ =0.32	0.58
	Repertoire Novelty	2.65 ± 0.17	2.24±0.49	$F_{1,50}$ =0.63	0.43	2.70±0.09	2.79±0.17	$F_{1,83}$ =0.25	0.62
	No. of Unique Songs	5	0			3	1		
	Sample Size	8	1			12	3		
Overall	Repertoire Size	6.96±2.26	6.75±1.71	$F_{1,51}$ =0.01	0.93	11.55±0.27	12.50±0.89	$F_{1,84}$ =1.05	0.31
	Song Sharing	0.41±0.02	0.40±0.05	$F_{1,50}$ =0.08	0.78	0.47±0.01	0.47±0.03	$F_{1,83}$ =0.00	0.99
	Repertoire Novelty	3.21±0.08	2.99 ±0.26	$F_{1,50}$ =0.64	0.43	2.53±0.04	2.63±0.14	$F_{1,83}$ =0.06	0.81
	No. of Unique Songs	19	1			26	4		
	Sample Size	53	4			84	5		

Table 4.3: Mean (± SE) values of five fine structural measurements of male and female resident and first-generation migrant solo songs. Statistics presented are for the results of the MANOVA analyzing the five song variables. Male and female first-generation migrants and residents showed no significant differences in acoustic structure (p>0.05).

		Females				Males			
Population	**Song Trait**	**Resident**	**First-generation Migrant**	**$F_{1, 48}$**	**p**	**Resident**	**First-generation Migrant**	**$F_{1,79}$**	**p**
Santa Rosa	Duration of song (s)	1.96±0.04	1.78±0.18	0.94	0.34	2.11±0.03	-	-	-
	Trill Rate (elements/s)	11.67±0.56	10.28±2.27	0.35	0.56	10.86±0.23	-	-	-
	Dom. Freq. of Trill (Hz)	1057±14	1072±56	0.07	0.80	886±4	-	-	-
	Max Frequency (Hz)	2593±68	2145±276	2.48	0.12	1999±36	-	-	-
	Min Frequency (Hz)	917±17	904±69	0.34	0.85	818±4	-	-	-
Rincon	Duration of song (s)	1.81±0.08	1.65±0.25	0.36	0.55	1.95±0.05	1.87±0.13	0.28	0.60
	Trill Rate (elements/s)	10.50±1.07	10.43±3.21	0.00	0.98	10.23±0.46	10.16±1.18	0.00	0.95
	Dom. Freq. of Trill (Hz)	1190±26	1188±79	0.00	0.98	885±8	862±19	1.25	0.27
	Max Frequency (Hz)	2591±130	2437±390	0.14	0.71	1824±73	1686±185	0.48	0.49
	Min Frequency (Hz)	1064±32	1104±98	0.15	0.70	785±8	756±20	1.56	0.22
Monteverde	Duration of song (s)	2.15 ±0.09	2.28±0.25	0.24	0.63	2.20±0.05	2.35±0.11	1.79	0.18
	Trill Rate (elements/s)	8.16±1.14	6.70±3.21	0.19	0.67	9.22±0.46	8.38±0.96	0.62	0.43
	Dom. Freq. of Trill (Hz)	1109±28	1152±79	0.27	0.61	912±8	912±16	0.00	0.99
	Max Frequency (Hz)	2606±138	2604±390	0.00	0.99	2345±73	2132±151	1.60	0.21
	Min Frequency (Hz)	1062±35	1121±98	0.32	0.57	768±8	770±16	0.01	0.91

Figures

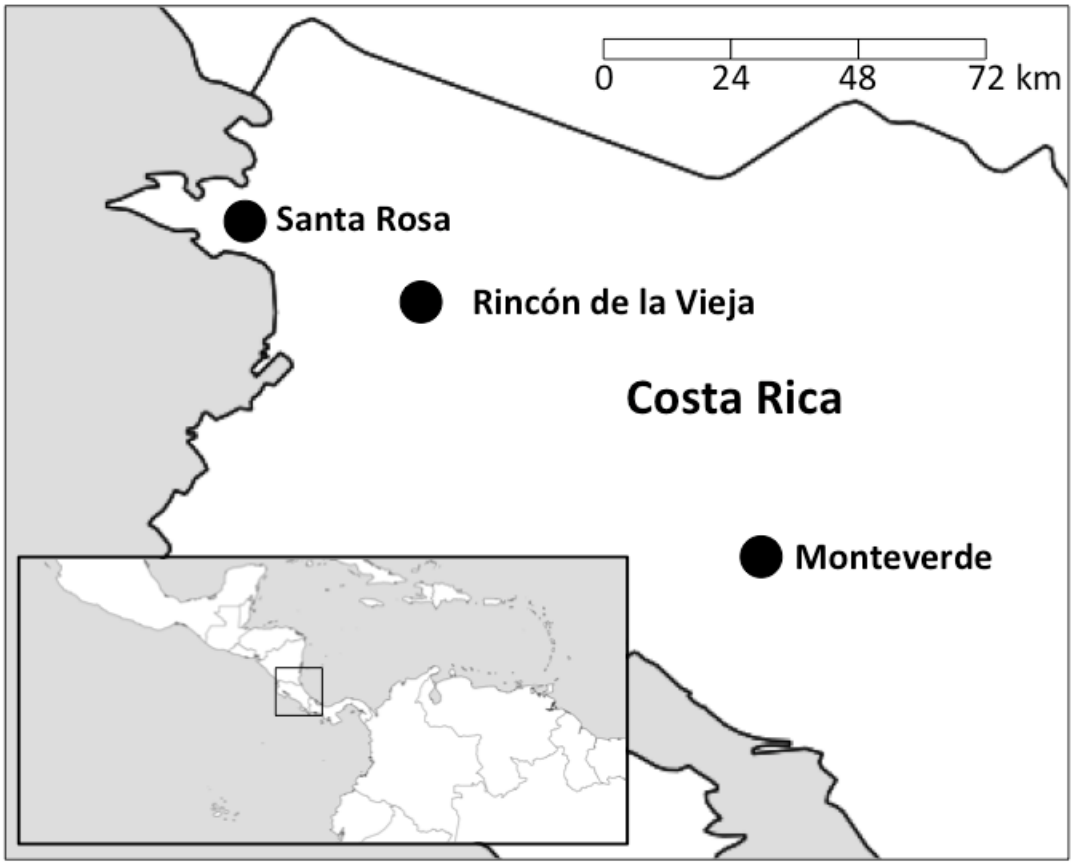

Figure 4.1: Map of the three populations of Rufous-and-white wrens in Costa Rica where genetic and acoustic samples were collected for comparisons of acoustic variation between residents and first-generation migrants. Inset shows map of Central and South America.

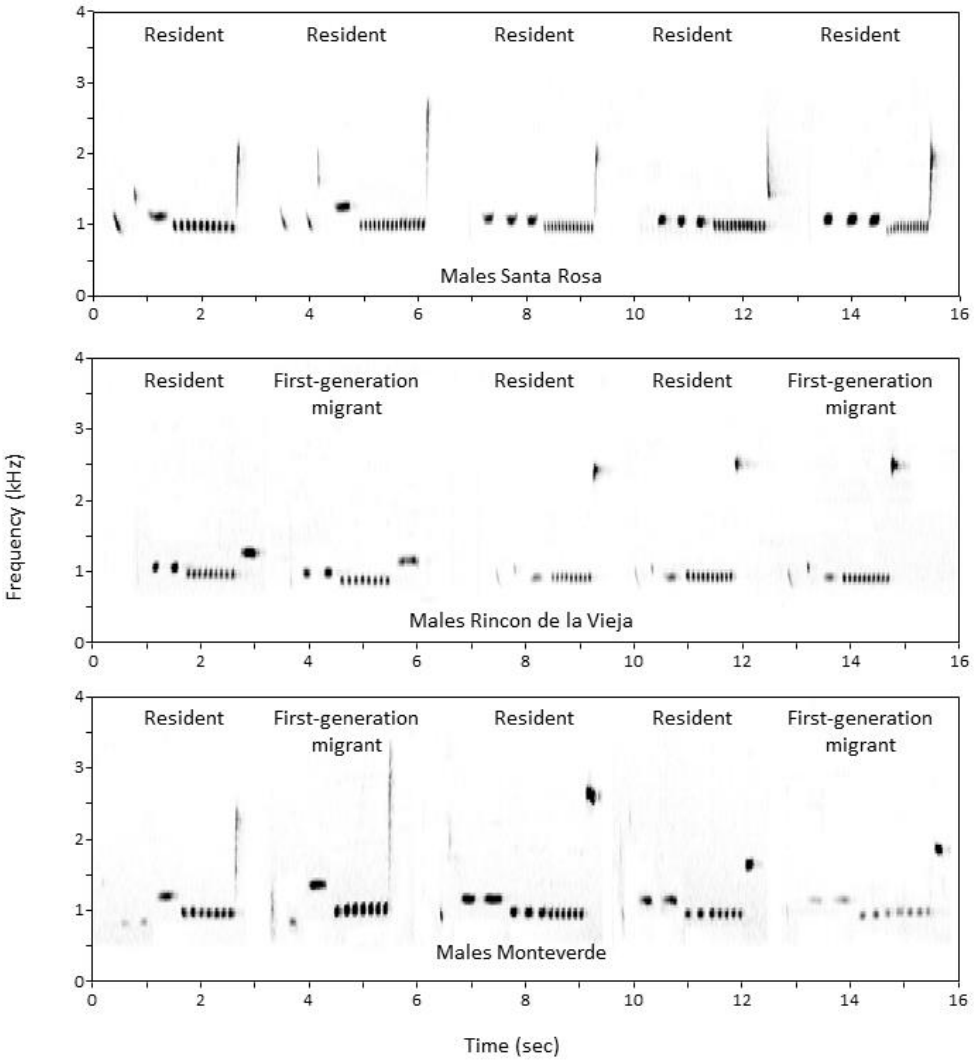

Figure 4.2: Sound spectrograms of example male Rufous-and-white Wren songs from birds identified as first-generation migrants and residents using GENECLASS 2.0 at our three focal populations.

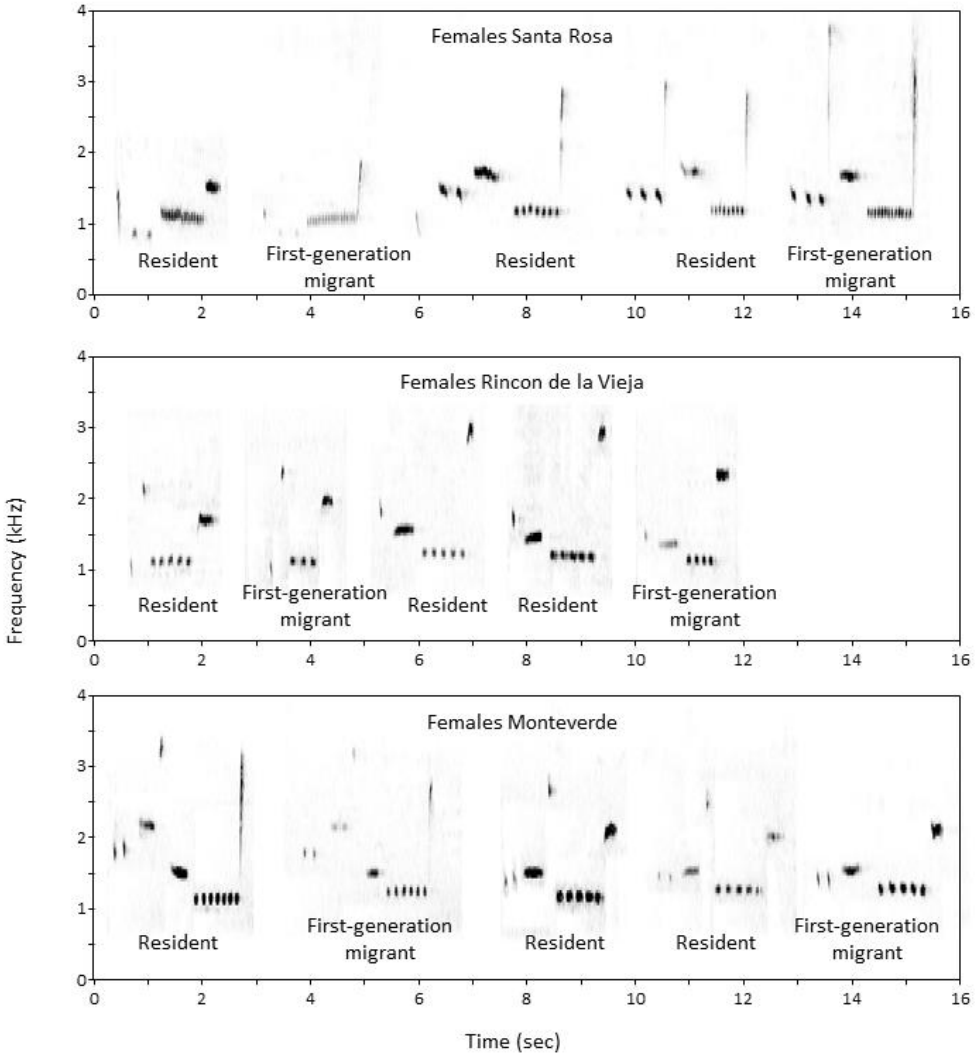

Figure 4.3: Sound spectrograms of example female Rufous-and-white Wren songs from birds identified as first-generation migrants and residents using GENECLASS 2.0 at our three focal populations.

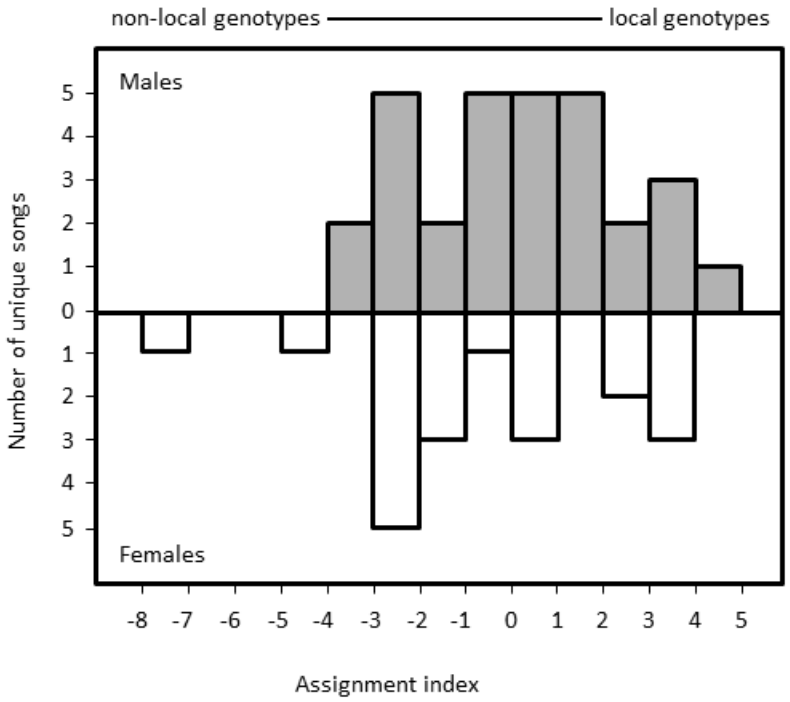

Figure 4.4: Combined frequency distribution of corrected assignment indices of Rufous-and-white Wrens who produced unique songs at our three focal populations. Distribution shows no significant difference between the number of unique songs produced by birds with negative assignment indices (i.e. non-local genotypes) and positive assignment indices (i.e. local genotypes). Male distributions are represented by the bars above zero, while female distributions are represented by the bars below zero.

Supplementary information accompanying Chapter 4

Table 4.S1: Population comparisons (mean ± standard error) of the vocal repertoires (repertoire size, song sharing and repertoire novelty) of male and female Rufous-and-white Wrens at each of our three focal populations. Statistics presented are for each population and overall from ANOVA (repertoire size) and ANCOVA (song sharing and repertoire novelty) analyses. Males and females showed significant differences in vocal repertoires among the three focal populations ($p<0.05$)

	Females					**Males**				
	Santa Rosa	**Rincon**	**Monteverde**	**Test**	**p**	**Santa Rosa**	**Rincon**	**Monteverde**	**Test**	**p**
Repertoire Size	7.21±0.36	6.60 ± 0.70	6.11±0.73	$F_{2, 54} = 1.04$	0.36	10.75±0.25	12.53±0.50	11.47±0.50	**$F_{2, 86} = 5.24$**	**0.007**
Song Sharing	0.25±0.02	0.34 ± 0.05	0.61±0.04	**$F_{2, 50} = 33.71$**	**<0.001**	0.48±0.03	0.51±0.02	0.43±0.02	**$F_{2, 82} = 3.89$**	**0.02**
Repertoire Novelty	3.91±0.13	2.89 ± 0.26	2.59±0.20	**$F_{2, 50} = 4.26$**	**0.02**	2.52±0.11	2.37±0.09	2.72±0.09	**$F_{2, 82} = 4.26$**	**0.02**

Table 4.S2: Population comparisons of the fine scale structural measurements of female and male songs. Values are presented as mean ± SE and statistics presented are for the results of the MANOVA analyzing the five song variables. Males and females showed significant differences in their acoustic structure of solo songs among the three focal populations ($p<0.05$)

	Females					Males				
	Santa Rosa	**Rincon**	**Monteverde**	**$F_{2,48}$**	**p**	**Santa Rosa**	**Rincon**	**Monteverde**	**$F_{2,78}$**	**p**
Duration of song (s)	1.87±0.09	1.73±0.13	2.22±0.13	**3.79**	**0.03**	2.11±0.03	1.91±0.07	2.28±0.06	**8.46**	**<0.001**
Trill Rate	10.98±1.17	10.47±1.69	7.43±1.70	1.53	0.23	10.86±0.23	10.20±0.63	8.80±0.53	**6.48**	**0.002**
Dom. Freq. of Trill	1065±29	1189±42	1131±42	3.16	0.05	886±4	873±10	912±9	**4.93**	**0.01**
Max Frequency (Hz)	2369±142	2514±206	2605±207	0.49	0.62	1999±36	1756±99	2239±84	**7.05**	**0.002**
Min Frequency (Hz)	910±36	1084±52	1091±52	**6.02**	**0.005**	818±4	772±11	769±9	**18.56**	**<0.001**

Chapter 5: Temporal variation in both male and female songs is correlated with cultural drift and not genetic drift in a long-term study of tropical wrens*

*This work is the outcome of joint research with D. Heath, R. Walter, and D. Mennill

Chapter Summary

Acoustic divergence is thought to play an important role in speciation because of its role in mating systems and territory defence. Therefore, by studying the forces that drive acoustic divergence we can gain deeper insight into the process of evolution. Many studies have focused on male song, but little is known about the evolutionary forces that drive the evolution of female song. Here we examine patterns of temporal variation in the songs of both male and female songbirds, to better understand the evolution of acoustic signals. We recorded songs from both male and female Rufous-and-white Wrens (*Thryophilus rufalbus*) in Costa Rica, and compared song type richness, song type abundance, and acoustic structure over an eleven-year period (2003-2013). We combined acoustic analyses with genetic analyses to determine if acoustic variation corresponds with genetic variation, as predicted by the Genetic Adaptation Hypothesis. We found that while song type richness and song sharing were consistent across years for males and females, acoustic structure and song type abundance patterns changed over time for both sexes. Although cultural patterns changed for both males and females, females exhibited greater cultural differentiation over time than males. Furthermore, we found that the distribution of allele frequencies changed over the same time period. While the two phenomena are occurring simultaneously, genetic and acoustic changes are occurring independently, as proposed by the Drift Hypothesis. Our results suggest that cultural drift influences cultural variation in both males and females. Differences between male and female cultural patterns likely reflect behavioural, life history, and selection differences between sexes, such as singing and dispersal differences. Our study adds to a growing body of work describing the cultural evolution of avian acoustic signals, but is the first study to explore cultural variation in female songbirds, and further emphasizes the acoustic and cultural differences that exist between male and female songbirds.

Introduction

Similar to biological traits, the acoustic signals of animals often vary spatially and for some species can be transmitted to other individuals via cultural learning (Lynch, *et al.*, 1989; Whiten *et al*., 2011). While much emphasis has been placed on learning and its influence on the evolution of behaviours, other factors work in unison with learning patterns to influence cultural evolution, including genetic changes and ecological influences (Laland and Janik, 2006). Cultural evolution is often compared to genetic evolution, because they are subject to the same evolutionary forces (drift, mutation, migration, and selection; Lynch, 1996). The question remains, however, are cultural changes linked to genetic changes, or do cultural changes occur independently of genetic changes? Studying the evolution of learned acoustic signals together with genetic patterns will help to further determine the role of behavioural traits during speciation (Edwards, 1993; Irwin *et al*., 2001).

Two key hypotheses that have been proposed to explain cultural evolution are the Genetic Adaptation Hypothesis (Marler and Tamura, 1964) and the Drift Hypothesis (Andrew, 1962). The Genetic Adaptation Hypothesis suggests that genetic and cultural evolution occur together, as a result of young animals learning vocal signals (or preferences for vocal signals in their natal territories), and then use vocal signals as a cue for assortative mating (Marler and Tamura, 1964). The Drift Hypothesis suggests that genetic and cultural evolution are not linked and that cultural evolution occurs as a result of the vocal learning process, regardless of whether populations are genetically different or not (Wright *et al*., 2005; Leader *et* al., 2008; Yoktan *et al.*, 2011). To date though only a handful of studies have demonstrated a direct relationship between acoustic and genetic divergence in animals that exhibit vocal learning (Baker *et al*., 1982; MacDougall-Shackleton and MacDougall-Shackleton, 2001).

The acoustic signals of birds have received considerable attention in the study of cultural evolution, given that birds may exhibit either innate and learned acoustic signals (e.g. suboscine

versus oscine Passeriformes repectively), making them a model system for studying the evolution of culture (Catchpole and Slater, 2008; Irwin, 2012). To date a number of long-term studies have examined how the acoustic signals of birds evolve over time (e.g. Ince *et al*., 1980; Payne *et al*., 1981; Gammons and Baker, 2005; Wright *et al*., 2008; Byers *et al*., 2010; Goodale and Podos, 2010; Azar *et al.*, 2014; Williams *et al*., 2014; García *et al*., 2015). These studies have examined acoustic signals over both short time periods (<10 years) and long time periods (up to 30 years), but have examined acoustic patterns independent of genetic patterns. Incorporating genetic variation in studies of culture can help to further determine the role of genetic variation in the evolution of culture (Laland and Janik, 2006). Additionally, genetic analysis can be used to measure other factors, including immigration, population size, and the presence of founder effects, which affect the evolution of culture (Parker *et al.*, 2007; Stewart and MacDougall-Shackleton, 2008; Potvin and Clegg, 2015).

To date the study of cultural evolution in birds has primarily focused on male acoustic signals, and the majority of these studies have been conducted at temperate latitudes (Riebel, 2003; Podos and Warren, 2007). While female song is uncommon at temperate latitudes, it is the ancestral state in birds, and is common and widespread in the tropics (Slater and Mann, 2004; Odom *et al*., 2014). Here we examine cultural evolution in a songbird, the Rufous-and-white Wren (*Thryophilus rufalbus*), a year-round resident of Central and South America. Both male and female Rufous-and-white Wrens sing and possess song repertoires (individuals can learn up to 15 songs; Mennill and Vehrencamp, 2005; Harris *et al*., 2016). Given that both males and females sing a variety of different song types, it allows us to track song types through time and compare patterns of cultural evolution between sexes.

The goal of our study was to examine whether male and female songs change through time by studying three cultural traits: song type richness (i.e. the number of song types present in the

population), song type abundance (i.e. the frequency with which song types are sung), and acoustic structure (i.e. the spectral and temporal measurements of songs; see glossary). We combined acoustic and genetic analyses to examine the role of cultural and genetic drift on the evolution of male and female song over an 11-year period in a population of Rufous-and-white Wrens in Costa Rica. We incorporated genetic analysis to measure the level of genetic diversity and genetic differentiation across time, to compare dispersal differences between sexes, and to identify potential immigrants in our population. If cultural traits are correlated with genetic variation, this would suggest a role for genetic drift in the evolution of male and female songs as predicted by the Genetic Adaptation Hypothesis (Marler and Tamura, 1964). If, however, cultural traits are not correlated with genetic variation, this would suggest that cultural drift and cultural selection have a greater influence on the evolution of male and female songs, as predicted by the Drift Hypothesis (Andrew, 1962). We compared patterns of temporal variation between sexes, to better determine whether males and females exhibit different patterns of cultural evolution.

Glossary

Acoustic structure: fine-structural characteristics of song (i.e. based on measurements of sound spectograms).

Assignment Index: probability of a genotype originating in the population from which it was sampled. Individuals with low and negative values are less likely to have been born in the population where they were sampled, whereas individuals with a high positive value are more likely to have been locally recruited (Paetkau *et al*., 1995).

Cultural diversity: diversity of cultural traits (song types) in a population. Cultural diversity takes into account the total number of cultural traits that are present and the distribution and frequency of each cultural trait in the population.

Cultural drift: variation in the frequency of cultural traits (songs or syllables) due to random differences in the way songs are learned or produced. Cultural traits may change in structure through copying errors or improvisation (e.g. changes in the frequency or duration of a song type) or how frequently they are produced in the population (e.g. old song types may disappear and be replaced by new song types).

Cultural evolution: changes in socially transmitted behaviours (e.g. songs) over time in a process that mirrors biological evolution.

Cultural transmission: the learning of songs by individuals from conspecific birds.

Drift Hypothesis: predicts that cultural and genetic evolution are not linked, and while cultural groups may be genetically distinct, cultural differences arise as a result of the song learning preferences or cultural drift.

Genetic Adaptation Hypothesis: predicts that cultural and genetic evolution evolve in tandem, as a result of young birds learning songs in their natal areas, and then using song as a cue for assortative mating.

Genetic diversity: the diversity of alleles in a population. Genetic diversity takes into account the total number of alleles that are present and the distribution and frequency of occurrence for each allele in a population.

Genetic drift: variation in allele frequencies in a population due to chance. Genetic changes are not associated with fitness, but result from random differences in the survival and reproduction of individuals.

Song type abundance: the frequency with which a song type is present in the population.

Song repertoire: the collection of song types that have been learned by a single individual

Song sharing: proportion of song types shared between two or more individuals

Song type: songs containing a series of notes that are combined in a stereotypical order.

Song type richness: the number of different song types present in a population.

Temporal variation: within-population differences in cultural and genetic traits across time.

Methods

From 2003 to 2013 we studied a population of Rufous-and-white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area (10.85 °N, 85.60 °W) in northwestern Costa Rica. Each year we captured birds using mist-nets, and banded individuals with a unique band combination that included three colour bands and a numbered aluminum band. From each bird we collected a small blood sample (~100 μl) from the brachial vein, and stored blood samples in Queen's Lysis buffer or 95% ethanol. We determined sex based on the presence of a brood patch (females) and by singing behaviour (sexes can be distinguished based on fine-structural differences in songs; Mennill and Vehrencamp, 2005).

Over this 11-year period, we recorded birds annually from April to July, a period of high vocal output for this species (Topp and Mennill, 2008). The majority (approximately 60%) of our recordings were collected during focal recordings, where we followed individually marked birds around their territories for 60 to 90 minutes each morning (from 0445h to 1100h) and confirmed the birds' identities by resighting colour bands during the recording. We recorded each individual at least twice during focal recordings each year using a solid-state digital recorder (PMD-660 Marantz or PMD-661 Marantz; 44.1 kHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70 or ME67/K6). We supplemented focal recordings with recordings from automated recorders (see Harris *et al*., 2016 for details). We placed these recorders within the center of the territories of each focal pair, often within 10m of the focal pair's nest. We confirmed that the songs collected by these automated recorders were those of the intended pair by re-sighting the focal individuals in their territory after automated recording sessions, and by matching the songs collected by the automated recorders to the songs collected during focal recordings (Harris *et al.*, 2016).

Song type assignment

We annotated all audio files using SYRINX-PC sound analysis software (J. Burt, Seattle, Washington, USA) and built a library of all the song types in the repertoire of each male and female (134 males and 103 females). We classified songs based on their spectro-temporal properties, following the approach outlined in Harris *et al*. (2016). Previous work in this study population by Barker (2008) has shown that discriminant analysis can differentiate song types based on fine-structural measurements (i.e. duration of song, maximum frequency, minimum frequency and inter-syllable interval), and we incorporated these methods to help assign song types correctly. We used the following criteria to determine whether a song was shared between two individuals: (i) songs shared the same sequence of elements in the introductory part of the song (although we ignored differences of one or two syllables in this section because first notes are produced quietly and sometimes difficult to detect); (ii) introductory syllables were produced at the same frequency (within100 Hz); (iii) trills were composed of the same type of syllables (i.e syllables were the same length and shape); (iv) trills were produced at the same frequency (within 100 Hz); (v) trills were delivered at the same rate (within two elements / s); and (vi) terminal syllables were the same shape (e.g. long tonal syllables that covered a short bandwidth versus short syllables with a broad bandwidth).

Measurements of cultural diversity

To quantify cultural diversity, we measured song type richness and song type abundance within and across years. We quantified song type richness (i.e. the total number of songs in the population) for each sex by counting all of the song types recorded within each year, and compared patterns between sexes using an analysis of variance (ANOVA). To compare patterns of song type richness across years for each sex we calculated Chao-2 indices for males and females each year, using the software package estimateS 9.1.0 (Colwell, 2013). We used extrapolation and rarefaction

techniques to estimate song type richness, treating each song type as a distinct unit. This approach is primarily used to estimate the number of species, but has been used effectively to estimate syllable diversity in populations of Island songbirds (Potvin and Clegg, 2015). We used this approach to complement our other measures of diversity, because not only does it account for sample size differences, but is especially reliable for estimating diversity when some classes are under represented (Chao, 1984), thereby allowing us to make comparisons of diversity among years. We estimated the number of song types using 1000 randomizations without replacement. We chose this number as it was approximately double the highest number of song types that we recorded in a year (n=562 in 2012). We then plotted rarefaction-extrapolation curves with 95% confidence intervals for each year for each sex to compare patterns of song type richness across years for both sexes.

We calculated repertoire sharing among individuals within years and across years, using an adjusted Jaccard's coefficient (S_j) of sharing with the following formula (Tracey and Baker 1999):

$$S_j = c/((a+b+c)-d)$$

where a = the number of songs in individual A's repertoire but not individual B's, b = the number of songs in individual B's repertoire but not individual A's, c = the number of songs shared between two individuals, and d = the difference in repertoire size between individual A and B. For this value we present the average percentage of songs that an individual shares with all members of the same sex within the study population. We performed an ANOVA to compare patterns of within-year song sharing within and between sexes.

To further compare patterns of song type abundance, we calculated cultural distance between years. The purpose of this was to determine whether song frequencies change with time (i.e. whether the same songs continue to be learned with the same frequency across time). We calculated Morisita indices of sharing between years. We used this index to account for how common a specific song type was within each year, as opposed to whether or not it was present

(like that of the Jaccard's modified index used above). Given that this index quantifies sharing, we subtracted the calculated Morisita index value from 1 to measure cultural distance between two time periods. We did this for each sex, and compared patterns of cultural distance between sexes using a Mann-Whitney U test.

Acoustic structure

To quantify variation in the songs of male and female Rufous-and-white Wrens we collected ten different temporal and spectral measurements of their songs. For each song we measured: (i) the duration of the song (s), (ii) the number of syllables, (iii) element rate of the trill (the number of elements/second in the trill portion of the song), (iv) dominant frequency of the trill (Hz), (v) length of the terminal syllable (s), (vi) bandwidth of the terminal syllable (Hz), (vii) dominant frequency of the terminal syllable (Hz), (viii) duration of all the pauses in the song (s; we considered a pause as the space between the end of one syllable and the beginning of the next syllable), (ix) minimum frequency of the song (Hz), and (x) maximum frequency of the song (Hz). We used the automated parameter measurements tool in AviSoft-SASLab Pro (version: 5.2.04; R. Sprecht; Berlin, Germany) to measure these features, thereby minimizing subjectivity in the fine-structural measurements. Songs were resampled to 8000 Hz, which allowed maximum spectral resolution (the maximum frequency of Rufous-and-white Wren songs in this dataset was less than 4000 Hz). For each song we created a spectrogram with an effective resolution of 8Hz and 4 ms (settings: transform size: 1024 Hz; overlap: 96.86%; window: Hamming). We used a high-pass filter of 500 Hz to remove any low-frequency background noise from the sound files.

For males we measured 1327 songs representing 10 different song types from 61 different males (average number of songs measured/male= 21.75 songs, range=7-46). For females we measured 406 songs representing 35 different song types from 51 females (average number of songs measured/individual= 7.96 songs; range=1-26). We measured the fine structure of songs from

three different temporal periods: 2003, 2007, and 2013 (the three temporal points were chosen based on genetic analyses that found 2003 and 2013 to be significantly different between from each other, while 2007 was not significantly different from either 2003 or 2013 based on genetic analysis; see Results). In one instance a female was recorded in both 2007 and 2013, so we measured songs from her in both 2007 and 2013. Whenever possible we included songs from multiple recordings (i.e. from a different day), and measured up to three exemplars for each song type per recording. All measurements used for all statistical analysis represent means for each individual bird. To account for differences in sampling, we calculated an average value of all the songs that we measured for each individual; we then used the average of these measurements for each of the ten variables listed above. This gave rise to measurements for 310 male and 206 female songs in our analyses. Last we tested for intercorrelations between all fine-structural variables using a Pearson correlation analysis; none of the intercorrelations (r) exceeded 0.7 and therefore we included all 10 variables in our analyses (Ruegg *et al*., 2006). We tested all fine-structural variables for normality using a Shapiro-Wilks test and by inspecting Q-plots of the residuals.

To better understand inter-annual variation in song types, we used several different approaches. First we conducted a Principal Component Analysis (PCA) to reduce the measurements into fewer metrics for our analysis. We analyzed males and females separately, and performed our PCA on all male songs and female songs that we measured. We ran a separate PCA for each sex with direct oblimin rotation, because this method allows for correlations between components, and retained all principal components with Eigenvalues above 1.0. The first three principal components explained 75.1% and 70.2% of the variance for male and female song types respectively (Table 5.S1). To determine if songs varied between years we performed a Multivariate Analysis of Variance (MANOVA) on the three retained principal components, using principal component scores as our dependent variable and year as our independent variable. Finally we used a *k*-means analysis,

following the approach of Goodale and Podos (2010), to determine if songs were more similar to each other within years than across years. Again, we analyzed the first three principal components for both male and female song types as our dependent variables, and ran our analysis at *k*=3 to test if songs could be distinguished based on the year they were recorded.

We performed MANOVA on a subset of song types to test if song types varied among years for our ten fine-structural measurements. Again comparisons were made between our three temporal periods: 2003, 2007, and 2013. For this analysis we focused exclusively on four male and three female song types (Figure 5.1). We selected these song types because they are well-represented across the time span we investigated, and because two of the song types (song types 2 and 3 in Figure 5.1) are sung by both male and female Rufous-and-white Wrens. Given that our analyses focused on temporal differences in song types within each sex, we performed a separate MANOVA for each of the four male song types and three female song types, where each of the ten fine-structural measurements were included as the dependent variable, while year was set as our independent variable. To compare variation in song types between sexes, we calculated the coefficient of variation for each of the fine-structural measurements and compared the level of variation between sexes using a Filgner-Kileen test to evaluate the null hypotheses that variance within song types is the same between sexes (Donnelly and Kramer, 1999). For this analysis we focused exclusively on the two song types (song type 2 and 3, Figure 5.1) that are sung by both males and females.

Microsatellite genotyping and analyses

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega), and genotyped 213 individuals (123 males and 90 females) at 10 microsatellite loci. We used four previously designed microsatellite loci primer sets *Th-Pl 14, Th-Pl 20, Th-Pl 30* (Brar *et al*., 2007), *RWWR 2c* (Herman Mays personal communication), and developed six new microsatellite primer

loci sets (*Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, and Tru 25;* Table 3.S1) following the microsatellite enrichment procedure detailed in Walter *et* al., (2007). All PCR reactions were conducted in 12.5 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 1.25 μL of 10x PCR buffer (Applied Biosystems), 0.5 μL of $MgCl_2$ (2.5 mM), 0.45 μl of dNTPs (0.2 mM), 0.05 μl of bovine serum albumin, and 0.5 U of Taq (Genscript, Applied Biosystems). For the primer sets *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25,* and *RWWR 2c,* we included 1 μM each of the tel-forward, reverse and M13 dye-labeled primer (GTAAAACGACGGCCAGT). For the remaining three primer sets (*Th-Pl 14, Th-Pl 20,* and *Th-Pl 30*) we used 1 μM each of the forward primer and the IR-dye labeled reverse primer. PCR conditions for *Th-Pl 14, Th-Pl 20,* and *Th-Pl 30* followed those described in Douglas *et al*. (2012), while for the remaining primer sets we used the following PCR conditions: one cycle of 94.0°C for 2 minutes, followed by 34 cycles of 94.0 °C for 10 seconds, 50.0°C for 10 seconds, 72.0°C for 30 seconds, followed by a final extension cycle of 72.0°C for 90 seconds, although for the primer set *Tru 24* we increased the annealing temperature (T_2) to 54.0°C to eliminate stuttering. PCR products were visualized on a 6% acrylamide gel on a Licor 4300 DNA analyzer; to ensure consistent sizing and scoring across gels, we ran controls with known size standards on each run. Allele sizes were scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

We tested for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium in GenePop version 4.0.10 (Raymond and Rousset, 1995), and corrected for multiple tests using sequential Bonferroni corrections (Rice, 1989). We calculated allelic richness (A_R) and inbreeding coefficient (F_{IS}) using FSTAT version 2.9.2.3 (Goudet, 1995), and calculated observed (H_O) and expected heterozygosity (H_E) in GenALeX 6.501 (Peake and Smousall, 2006; 2012). We compared genetic diversity patterns within years and among years, using Kruskal–Wallis tests, and between sexes using Mann-Whitney U tests. Finally we estimated effective population size (N_e) using the moment technique in Ne Estimator V2.01 (Do *et al*., 2014), using the linkage

disequilibrium method (Waples and Do, 2010), with a critical value of 0.02, where only those alleles that occurred with a frequency above this value were used to estimate effective population size. This method assumes no migration, but has been shown to give accurate estimates of effective population size (Gilbert and Whitlock, 2015). The purpose of estimating effective population size in this study was to quantify population size over the length of our study, given the potential effect of founder effects and bottlenecks on cultural patterns (Lynch, 1996; Potvin and Clegg, 2015).

We tested for differences in allele frequency distributions among years using pairwise exact tests in GenePop (1000 dememorization steps for 10,000 iterations). Multiple tests were corrected for using sequential Bonferroni corrections. To visualize and further assess genetic differentiation across years we used the Discriminant Analysis of Principal Components (DAPC; Jombart *et al*., 2010) method using the ADEGENET 2.01 package (Jombart and Ahmed, 2011) in R 3.2.3 software(R Core team, 2014). DAPC transforms the data using principal components and then preforms discriminant analysis on the retained principal components. For our analysis, we retained the first 70 principal components, and the first two discriminant functions.

To identify potential immigrants or individuals with uncommon genotypes we used partial Bayesian genotype assignment (Rannalla and Mountain, 1997) in GENECLASS 2.0 (Piry *et al*., 2007). Using Monte Carlo resampling (10,000 replicates; Paetkau *et al*., 2004) we excluded individuals from our focal population if they had a probability of less than 0.05 of originating from our study population. For this analysis, we compared all individuals from each year (2003-2013) separately, to 114 individuals from four other nearby populations (see Chapter 2 for details). This approach allowed us to estimate the number of immigrants that were present in each year. Additionally we generated genetic assignment indices for each individual by comparing individuals across all years with one other. This allowed us to identify the individuals with uncommon genotypes in our study site over the duration of the study.

Correlation between acoustic and genetic patterns

To determine if cultural diversity is linked to genetic diversity, we ran a linear regression model, using song richness as the dependent variable. For this model, we used observed heterozygosity, sex, and year as our independent variables. The purpose of this analysis was to see if population level changes in song type richness are related to changes in genetic diversity (using observed heterozygosity as a proxy for genetic diversity). Next we tested if changes in song frequencies (i.e. whether the same songs are continuing to be learned over time) were linked with genetic changes and temporal changes. We measured the relationship between cultural distance, genetic distance, and temporal distance for each sex (across all 11 years), using Mantel and partial Mantel tests. Cultural distance was calculated as listed above, and we calculated temporal distance as the number of years between two time periods. We measured genetic distance between time periods by calculating pairwise Nei's genetic distance in GenAlEx.

We further examined the relationship between genetic distance and culture by comparing genetic distance with song sharing and song structure at the individual level. To quantify genetic distance, we used the individual genetic assignment index, which is the expected frequency of an individual's genotype originating from the population from which it was sampled (Paetkau *et al.*, 1995). Individuals with lower assignment indices have rare or uncommon genotypes, which may indicate that these individuals are recent immigrants. By comparison individuals with higher assignment indices have more common genotypes and are therefore likely to have been born in the population in which they were sampled from (Mossman and Waser, 1999).

To analyze within-year song sharing (song sharing among all individuals within a year), we used linear regression, and set within-year song sharing as our dependent variable, within-year genetic assignment index, and year as our independent variables, and individual identity as a random factor, because some individuals were present in multiple years (range: 1-8, average =3.05).

We performed this analysis using the "nlme" package in R 3.2.2 (Pinheiro *et al*., 2016). This allowed us to test if the birds with the most different genotype (based on their genetic assignment index) have the most different repertoires, and thereby potentially introduce new songs into the population. Last, we examined the relationship between acoustic structure and genetic distance. For this analysis we conducted a PCA on the seven individual song types we measured (Figure 5.1) to reduce the ten variables we measured to a single composite variable. We then plotted the first PC against individual assignment index values to determine if the birds with the most different genotype sang the most atypical songs relative to the rest of the population. We ran this analysis separately for each of the seven different song types we measured (four male and three female song types).

All statistical tests were conducted in SPSS (Version 23.0, SPSS Inc., Chicago, IL, USA) and PAST 3.11 (Hammer *et al*., 2001).

Results

Male and female cultural patterns

Song type richness was significantly higher in male Rufous-and-white Wrens than females (males: 39.55±1.37 song types; females: 35.00±1.37 song types; $F_{1,20}$=5.51, p=0.03; Table 1); we identified 69 distinct male song types, and 59 distinct female song types over the duration of our study. Although song type richness varied across years within each sex, the 95% confidence intervals for each year all overlapped (Table 5.1; Figure 5.2), indicating that song type richness estimates were comparable across the 11 years of our study. Overall each male song type was sung by 17.60±3.54 (range: 1-122) individuals, while a female song type was sung by 11.77±1.98 (range: 1-77) individuals over the 11-year period. Approximately one-third of all male song types (21 of 69) and female song types (20 of 59) were unique to a single individual. Seventeen male song types

(22.1%) and female song types (27.8%) were present in all years. On average, a male song type was detected for 6.10±0.41 years, while a female song type was detected for 6.52±0.52 years.

We analyzed song-sharing patterns across all years and within each year for both male and female Rufous-and-white Wrens. Overall, males shared more songs than females across the 11-year period (males: 0.46±0.01; females: 0.28±0.01; $F_{1, 503}$=622.02, p<0.001). Within-year song sharing significantly varied across time for both sexes, ranging from 0.39 to 0.50 ($F_{10, 290}$=5.57, p<0.001) for males and from 0.24 to 0.36 for females ($F_{10,188}$=5.68, p<0.001). For males, 4 of 45 post-hoc comparisons were significant following corrections for multiple comparisons, while 2 of 45 post-hoc comparisons were significant for females (Table 5.S2). Cultural distance (based on Morisita dissimilarity values) ranged from 0.02 to 0.09 between years for males, and ranged from 0.07 to 0.23 for females (Table 5.S3). Overall, cultural distance between years was significantly greater in females than males (males: 0.04±0.01; females: 0.12±0.01; *z*=-6.66, p<0.001).

Fine-structural measurements of songs

Across years, songs were not significantly different from each other based on our Multivariate Analysis of Variance on the first three principal components summarizing variation in 10 fine-structural measurements of songs for both sexes (males: Wilks' λ=0.99, $F_{6, 610}$=0.45, p=0.84; females: Wilks' λ=0.98, $F_{6, 402}$=0.82, p=0.56; Figure 5.3). Furthermore, when we analyzed the first three principal components using *k*-means clustering, we found no indication of temporal clustering for either males or females, given that each of the three clusters included songs from all three years included in the analysis (males: X^2=0.46, p=0.98; females: X^2=2.72, p=0.61). Overall our results indicate that the fine-scale acoustic structure of male and female song types was relatively stable across the 11-year period.

We found significant temporal variation across years for 3 of 4 male song types and 2 of 3 female song types (MANOVA: Males–song type 1:Wilks' λ=0.38, $F_{20,70}$=2.10, p=0.012; song type 3:

Wilks' λ=0.47, $F_{20,82}$=1.90, p=0.023; song type 4: Wilks' λ=0.19, $F_{20,62}$=4.02, p<0.001; Table 5.2; Females–song type 3: Wilks' λ=0.21, $F_{20,34}$=1.98, p=0.039; Table 5.3). For males, nine variables showed significant differences across years; three variables, trill rate, the duration of pauses in a song, and the dominant frequency of the trill were significantly different for multiple song types (2 of 3). For females three fine-structural measurements of song type 3 showed significant variation across years; trill rate, duration of pauses in a song, and dominant frequency of the trill all showed significant differences among years. Song length was also significantly different between years for female song type 1.

Two of the song types we measured (song type 2 and 3) were commonly found in the repertoires of both males and females in our population. Song type 2 did not show significant variation across years in our analyses of both male and female song types (i.e. MANOVA of song features across years), while song type 3 did show variation across years. Males and females showed differences in the variables that varied across years. Male songs varied significantly in length among years only, whereas female songs varied significantly in three fine-structural measurements: trill rate, duration of pauses in a song, and dominant frequency of the trill. Comparisons of inter-annual variation between sexes suggest that female songs exhibit greater inter-annual variation than males (Table 5.4). Although our analysis examined only two song types shared by males and females, females exhibited greater variation across years than males in both of these song types.

Genetic diversity

We genotyped 213 individual Rufous-and-white Wrens (124 males and 89 females); the ten microsatellites used in our study showed high variation across all years. Three of 110 tests showed deviations from Hardy-Weinberg Equilibrium, while 2 of 45 loci combinations showed evidence of linkage disequilibrium following corrections for multiple comparisons. We observed no significant differences in genetic diversity (A_R, F_{IS}, H_o, or H_E; Table 5.5) across years, or within each sex (P>0.17,

Kruskall-Wallis). Between sexes, only A_R was significantly different, with males showing greater allelic richness than females ($p<0.05$). Effective population size was relatively consistent across years, ranging between 171 and 289 in eight of the eleven years, and rose above 600 in 2003 and 2013. In 2004 the effective population was estimated as infinity. In this instance the program was likely unable to accurately estimate population size because we only genotyped 18 individuals that year.

We found significant differences in allele frequencies for 13 of the 55 pairwise comparisons (Fisher's exact tests: $p<0.001$). The first three years were significantly different from later years; 2003 was significantly different from all years from 2008-2013, 2004 was significantly different from both 2010 and 2011, while 2005 was significantly different from all years from 2009 to 2013. Similar to our results detected using Fisher's exact tests, DAPC suggested similar patterns of temporal genetic differentiation (Figure 5.4). Again the first three years (2003-2005) were more similar to each other than later years, while later years (2008-2013) clustered more closely together.

Genetic assignment analyses excluded 19 individuals (8.9%) from our study population across all years. Within each year between 1 and 4 individuals (range: 1.8 to 13.3%/year, Table 5.5) were excluded from our population based on genetic assignment, suggesting immigration occurs annually but at relatively low rates. Comparisons of dispersal between sexes revealed that females are the more dispersive sex (see Chapter 6). Assignment indices were significantly different between sexes, with females more likely to be immigrants than males (two-tailed t-test: $t_{1, 212}=1.72$, $p=0.09$; females-assignment index=-0.33; males-assignment index=0.24). Females had a negative assignment index for 7 of the 11 years, although assignment indices were significantly different between sexes for only a single year (2009; $t_{1, 48}=-2.21$, $p=0.03$), when females were the more dispersive sex (-0.97 versus 0.56 for males).

Correlation between song and genetic variation

Song type richness was not correlated with genetic diversity (*t*=-1.71, p=0.10), although our model was significant ($F_{1,20}$=13.31, adjusted r^2=0.37, p=0.002). In this model, sex was the only significant predictor of song type richness, where song type richness was higher in males than females (slope=-7.18±0.04, *t*=-3.65, p=0.002), whereas year did not significantly predict song type richness (t=-1.61, p=0.12). This result indicates that changes in song type richness were not related to changes in genetic diversity in our study.

We found that cultural distance was significantly correlated with genetic distance and temporal distance for both males and females, and that genetic distance was significantly correlated with temporal distance (Mantel test: r=0.69-0.92, p<0.001; Table 5.6). Our results, however, suggest that cultural drift is a greater driver of cultural patterns than genetic drift. Cultural distance and genetic distance were not significantly correlated when we controlled for temporal distance for either sex (partial Mantel test: males-r=0.33, p=0.76; females-r=-0.09, p=0.82). When we controlled for genetic distance, both male and female cultural distance significantly increased with temporal distance (partial Mantel test: males-r=0.70, p=0.001). Similarly when we controlled for cultural distance, genetic distance significantly increased with temporal distance (partial Mantel test: males-r=0.29, p=0.04; females-r=0.51, p=0.003).

Within years, song sharing was not correlated with genetic distance, indicating that males and females with more common genotypes did not exhibit greater sharing than birds with uncommon genotypes (males: adjusted r^2=0.01, p=0.39; t=0.68, p=0.50; females: adjusted r^2=0.02, p=0.04; t=0.16, p=0.89). Year was not a significant predictor of within-year song sharing for males (t=-1.03, p=0.30), but was a significant predictor for females (t=-2.01, p<0.05). Song structure was not significantly correlated with genetic distance for either sex. None of the seven models (one for each of the four male and three female song types we measured) we constructed were significant

(p>0.26), and neither assignment index (p>0.25), nor year (p>0.13) predicted the acoustic structure for the four male and three female song types we measured. Again these results suggest that the song structure of non-local birds is not significantly different from the song structure of local birds.

Discussion

Our investigation of cultural variation in Rufous-and-white Wrens adds to the growing list of studies that have demonstrated cultural evolution in the acoustic signals of birds, but is the first to examine the cultural evolution of female acoustic signals in birds. As in other studies, we observed temporal variation in the acoustic structure of song types, and the relative frequency of each song type in the population (Ince *et* al., 1980; Payne *et al.,* 1980; Harbison *et al*., 1999; Gammons *et al*., 2006; Wright *et al*., 2008; Goodale *et* al., 2010; Byers *et al*., 2010; Azar *et al*., 2014; García *et al*., 2015). We found this to be true for both males and females. Females exhibited greater cultural differentiation through time than males, indicating that the song types learned by females change more rapidly. In addition to demonstrating cultural evolution in our population, we also demonstrated genetic evolution. Drift appears to be the primary source of genetic change as opposed to immigration, given that we identified relatively few immigrants each year, and that the number of immigrants was fairly consistent over time. Overall our results imply that cultural drift is driving cultural evolution. Although cultural change and genetic change are occurring simultaneously, they appear to be occurring independently, as predicted by the Drift Hypothesis (Andrew, 1962). Below we discuss the role of drift, selection, and immigration in the cultural evolution of male and female Rufous-and-white Wren songs.

Males vs. Females

Although the overall patterns of cultural diversity for both sexes were consistent and stable within each sex over the duration of our study, they differed between the sexes. Song type richness and song type sharing were higher in males than females, while females exhibited greater changes

in song type frequencies across years than males. Males have larger repertoires than females (Mennill and Vehrencamp, 2005). Simulations run by Williams and Slater (1990) suggest that larger repertoires should result in lower sharing. Yet in contrast to Williams and Slater's predictions, male Rufous-and-white Wrens share more songs with other males than females do. These differences in cultural patterns between sexes may arise due to behavioural and neuroanatomical differences between sexes (Brenowitz and Arnold, 1986; Mennill and Vehrencamp, 2005). Male Rufous-and-white Wrens have larger song control regions than females, and repertoire size is correlated with the size of the song control region in this species (Brenowitz and Arnold, 1986). Furthermore, differences in singing behaviour are related to differences in the song control region (MacDougall-Shackleton and Ball, 1989); overall females sing fewer songs and less often than males (Topp and Mennill, 2006).

Dispersal differences between males and females may also affect cultural patterns. Females disperse farther from their natal territories as juveniles, and shift between breeding territories more often as adults (Chapter 6). Females had a lower assignment index overall than males on average, indicating that females are more likely to be immigrants than males. We did not observe any relationship between immigration and song sharing or song structure in either sex, although other studies have shown that immigrants introduce new songs into populations (Stewart and MacDougall-Shackleton, 2008; Fayet *et al.*, 2014). We found that females exhibit greater temporal variation than males within song types shared by both males and females. As a result of their higher dispersal rates, females share fewer songs with neighbours than do males, and female cultural patterns may therefore change more rapidly (Mennill and Vehrencamp, 2005; Chapter 6). Further research is necessary, but song types may evolve faster in females than males because female songs are more variable temporally than males, and this greater variation may be the result of their

dispersal capabilities, as has been suggested in other species where both sexes sing (Mennill and Rogers, 2006).

Another potential factor influencing sex-specific differences in cultural evolution is that males and females use their songs differently. The Social Adaptation Hypothesis predicts that birds learn songs to match those of their neighbours following settlement in a territory (Payne, 1981). To date this hypothesis has been applied almost exclusively to male songbirds (e.g. Yoktan *et al*., 2011). Hall *et al*. (2015) experimentally tested the function of female song in Banded Wrens (*Thryophilus pleurostictus*), a closely related congener, and found that female birds did not use their songs to countersing with rival females or attract mates. While songs may be used to defend territories, female responded to rival pairs in combination with their male partner. Hall *et al*. (2015) hypothesize that the main function for female song in this species is to communicate with their mate. If this is the case in Rufous-and-white Wrens, female songs may show greater variation and change faster, because females are not attempting to match their songs with rivals, and therefore sexual selection may not drive them to copy their neighbor's songs as accurately as males.

In duetting species, other selection pressures may influence temporal acoustic variation, because males and females combine their songs to produce duets. For instance Kōkako (*Callaeus wilsoni*) have been shown to choose partners from the same dialect, possibly because it is easier to produce duets with partners (Bradley *et al*., 2014). Additionally several duetting species adhere to duet codes, where males and females combine their songs non-randomly to produce duets (Logue 2006; Templeton *et al*., 2013) and the propensity to respond to an individual's mate in this manner may in turn restrict temporal acoustic variation. In this manner, selection may play a greater role in promoting song consistency, rather than promoting variation, but further studies are necessary to test this idea (Byers *et al*., 2010; Wilkins *et al*., 2013).

Relationship between cultural and genetic patterns

We found no relationship between cultural diversity and genetic diversity. Both cultural diversity and genetic diversity often decrease following reductions in population size as a result of bottlenecks or founder effects (Laiolo and Tella, 2007; Laiolo *et al*., 2008; Parker *et al.*, 2007). Estimates of effective population size in this study indicate that population size has remained relatively stable over this time period, likely contributing to the observed stable patterns of culture. In our population we observed changes in both cultural and genetic patterns over tim;, however, both male and female patterns of cultural evolution are not linked with genetic changes. At the population level, we did not observe a significant relationship between cultural distance and genetic distance when we controlled for time. Furthermore, individual genetic assignment indices do not predict acoustic structure or repertoire sharing. This suggests that the acoustic phenotypes of immigrants and residents are not significantly different; a pattern that we also observed at broader spatial scales (Chapter 3). In contrast to the Genetic Adaptation Hypothesis (Marler and Tamura, 1964), immigrants in our study appear to learn or adjust their adult songs post-dispersal (as predicted by the Social Adaptation Hypothesis; Payne, 1981),

Patterns of cultural evolution

Both the acoustic structure and the frequency of male and female song types in our population changed over time. Temporal variation may arise from two scenarios:(1) songs have changed structurally or been reorganized as a result of cultural drift, or (2) the distribution of song types has changed (i.e. some song types have become more common, while others have disappeared as a result of cultural extinction; Ince *et al*., 1980; Payne *et al*., 1981; Payne, 1996; Nelson *et al*., 2004; Byers *et al*., 2010; O'Laghlan *et al*., 2011). For example, Ince *et al*. (1980) found that cultural changes in Chaffinches (*Fringilla coelebs*) over an 18-year period resulted both from improper copying and extinction of rare song types (Williams and Slater 1990). While some song

types showed little variation across years, others were more variable, suggesting that song types evolve independently and at different rates (Nelson *et al*., 2004; Byers *et al*., 2010; García *et al*., 2015).

This raises the question: why do some song types survive longer than others? Payne *et* al. (1980) suggested that some songs are more easily copied or altered, and therefore survive longer and do not change in structure. Peters *et al*. (2012) found that acoustic structure may influence song type survival, because young Song Sparrows (*Melospiza melodia*) learn the least degraded songs, suggesting that some songs may not survive because they transmit poorly through the environment, and therefore cannot be heard and reproduced by young birds. In Rufous-and-white Wrens, female songs are generally more degraded over distance than male songs (Barker *et al*., 2009). Therefore, cultural differences between males and females may reflect transmission differences between male and female songs. Others may survive longer because they are used more often, resulting in young birds learning these songs from potential song-tutors (Wheelwright *et al*., 2009). In our study population some songs are more widespread throughout the population and are more commonly shared over time. These song types may survive longer because they are being produced more often and by both sexes, given that both males and females share song types. Therefore song types that are common in the repertoires of both sexes may create more opportunities for young birds to learn these song types.

Genetic Diversity

We observed significant genetic differentiation over time at our long-term study site. Changes in allele frequencies could result from genetic drift, migration, mutations, and selection. Given the strong relationship between temporal distance and genetic distance, our results emphasize the role that drift plays in influencing genetic differentiation, even over relatively short time periods. Tropical birds are known to exhibit strong patterns of philopatry (Stutchbury and

Morton, 2001), and the reduced levels of dispersal associated with philopatry and year-round territoriality not only promote genetic differentiation between populations but also increase speciation rates (Martin and Mckay, 2004; Clarumunt *et al*., 2012). Under this scenario, the limited dispersal of tropical birds and the associated reduction in gene flow enhance the effects of drift on genetic variation and differentiation (Francisco *et al*., 2007; Smith *et al*., 2014). The results of this study and our between-population analyses at broader scales demonstrate that while migration rates are relatively low, there is still gene flow (Chapter 2 and 3) between our study population and other nearby populations. Further long-term studies are necessary in tropical species, given that biodiversity is higher in the tropics. Studying long-term patterns of genetic variation at tropical latitudes will not only provide greater insight into the evolutionary processes that maintain this biodiversity, but also provide insight into how best to conserve this biodiversity (Moritz, 2002).

Conclusion

Our study demonstrates that cultural patterns are evolving independently of genetic patterns in male and female Rufous-and-white Wrens. Changes in cultural patterns are occurring despite song richness and acoustic structure remaining relatively stable over a 10-year time period, thereby suggesting that drift is playing a role in the temporal variation of song, a culturally-inherited trait. Similarly drift is an important driving factor of temporal genetic differentiation as well. Our results therefore provide further support for the Drift Hypothesis and highlight its importance during the speciation process. Additionally our work provides greater insight into the differences in the evolution of male and female acoustic signals. While males and females show differences in song diversity and cultural patterns, our study suggests that the acoustic signals of males and females are subject to similar driving forces.

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Tables

Table 5.1: Annual summary statistics of male and female song type diversity, song type richness, and song type sharing. Number of birds recorded (*N*); song richness (*S*) represents the total number of song types detected each year; Chao-2 is the estimated song type richness (± SD) accounting for unsampled song types; Song sharing represents the average within year song sharing percentage for each sex.

	Males					*Females*				
Year	***N***	***S***	***Chao-2***	***Chao-2 95% CI***	***Song Sharing***	***N***	***S***	***Chao-2***	***Chao-2 95% CI***	***Song Sharing***
2003	16	33	62.97±18.96	25.80-100.14	0.49	11	35	64.50±19.21	26.84-102.16	0.26
2004	20	35	56.60±11.01	35.01-78.19	0.50	18	40	74.89±20.77	34.17-115.60	0.24
2005	24	31	48.39±9.30	30.17-66.62	0.50	15	34	40.02±4.40	31.40-48.64	0.27
2006	22	38	52.92±8.00	37.24-68.60	0.48	18	37	45.93±6.73	32.75-59.12	0.34
2007	19	39	74.55±16.99	41.25-107.86	0.46	15	28	43.24±11.01	21.66-64.81	0.35
2008	25	39	79.62±15.30	49.64-109.60	0.46	19	35	50.94±10.19	30.97-70.91	0.27
2009	33	48	78.96±11.06	57.27-100.64	0.39	20	34	49.93±10.15	30.04-69.82	0.25
2010	29	46	79.47±12.45	55.07-103.87	0.41	14	32	57.08±17.76	22.28-91.88	0.36
2011	30	42	72.35±12.32	48.21-96.50	0.45	22	34	37.56±2.80	32.07-43.04	0.24
2012	47	46	55.96±4.35	47.44-64.49	0.49	24	39	47.09±5.81	35.69-58.48	0.26
2013	37	38	49.30±6.42	36.72-61.89	0.50	23	37	46.26±6.79	32.95-59.57	0.27

Table 5.2: Males exhibited temporal variation in the fine scale structure of songs based on the MANOVA of fine scale measurement of 10 variables. Song length is the total length of the song, Term length is the length of the terminal syllable; # Syllables is the total number of syllables in a song; Trill rate is the number of syllables in the trill per second; Pause length is the total duration of pauses in the song; Dom freq of trill is the dominant frequency that the trill is produced at; Dom freq of term syll is the dominant frequency that the terminal syllable is produced at; Term syll BW is the bandwidth that the terminal syllable covers; Max freq is the maximum frequency of the song; Min freq is the minimum frequency of the song; N is the number of individuals that were measured each year. All tests are significant at p<0.05.

	Song length (s)	Term length (s)	# Syllables	Trill Rate (syllables/s)	Pause length (s)	Dom freq of trill (Hz)	Dom freq of term syll (Hz)	Term syll BW (Hz)	Max freq (Hz)	Min freq (Hz)	N
Male Song Type 1: SR29											
2003	1.86±0.06	0.08±0.01	6.69±0.24	4.20±0.11	0.19±0.01	897±9	1328±38	81±14	1633±97	831±13	10
2007	1.96±0.05	0.08±0.01	6.82±0.18	4.08±0.08	0.20±0.01	878 ±7	1276±29	61±11	1667±75	843±10	17
2013	1.85±0.05	0.08±0.01	6.67±0.17	4.42±0.08	0.18±0.01	874±7	1387±27	91±10	1589±71	839±9	19
F	1.73	0.32	0.21	**4.59**	**3.46**	2.19	**3.89**	2.07	0.29	0.25	-
p	0.19	0.73	0.81	**0.02**	**0.04**	0.12	**0.03**	0.14	0.75	0.78	-
Male Song Type 2: SR9											
2003	1.97 ±0.07	0.04±0.01	12.89±0.51	10.53±0.40	0.08±0.04	89±19	2017±108	1479±139	2635±143	846±16	10
2007	2.15 ±0.06	0.04±0.01	13.36±0.42	10.48±0.33	0.09±0.01	863±16	2232±88	1423±113	2521±117	820±13	15
2013	2.21±0.04	0.04±0.01	14.51±0.31	10.66±0.24	0.09±0.01	904±11	2267±64	1515±83	2678±85	857±9	28
F	**4.62**	1.07	**4.79**	0.11	2.80	2.27	2.04	0.22	0.60	2.74	-
p	**0.01**	0.35	**0.01**	0.90	0.07	0.11	0.14	0.81	0.56	0.08	-
Male Song Type 3: SR4											
2003	2.21±0.07	0.06±0.01	17.24±0.74	14.15±0.46	0.08±0.01	898.36±9.89	1665.03±82.61	755±81	1967±78	812±12	13
2007	2.27±0.06	0.06±0.01	17.11±0.65	14.24±0.40	0.08±0.02	873.47±8.64	1727.48±72.24	918±71	2083±68	790±10	17
2013	2.22±0.05	0.06±0.01	17.56±0.50	15.24±0.31	0.08±0.01	869.15±6.74	1571.43±56.29	867±55	1925±53	808±8	28
F	0.40	0.23	0.17	2.90	1.98	3.10	1.52	1.16	1.72	1.23	-
p	0.68	0.80	0.85	0.06	0.15	0.05	0.23	0.32	0.19	0.30	-
Male Song Type 4: SR13											
2003	2.13±0.07	0.23±0.02	11.52±0.47	9.60±0.30	0.12±0.01	923±11	1183±38	44±11	1345±88	742±16	9
2007	2.23±0.06	0.23±0.02	12.49±0.40	10.14±0.26	0.12±0.01	879±9	1106±33	39±10	1379±76	766±14	12
2013	2.17±0.05	0.26±0.01	13.19±0.30	10.88±0.19	0.10±0.01	918±7	1119±24	34.96±7	1540±56	793±10	22
F	0.52	1.88	**4.67**	**7.16**	**12.63**	**6.94**	1.36	0.25	2.43	**3.71**	-
p	0.60	0.17	**0.02**	**0.002**	**0**	**0.00**	0.27	0.78	0.10	**0.03**	-

Table 5.3: Females exhibited temporal variation in the fine-scale structure of songs based on the MANOVA of fine scale measurement of 10 variables. Song length is the total length of the song, Term length is the length of the terminal syllable; # Syllables is the total number of

syllables in a song; Trill rate is the number of syllables in the trill per second; Pause length is the total duration of pauses in the song; Dom freq of trill is the dominant frequency that the trill is produced at; Dom freq of term syll is the dominant frequency that the terminal syllable is produced at; Term syll BW is the bandwidth that the terminal syllable covers; Max freq is the maximum frequency of the song; Min freq is the minimum frequency of the song; N is the number of individuals that were measured each year. All tests are significant at $p<0.05$.

	Song length (s)	Length of term (s)	# Syllables	Trill rate (Syllables/s)	Pause length (s)	Dom freq Of trill (Hz)	Dom freq of term syll (Hz)	Bandwidth of term syll (Hz)	Max freq (Hz)	Min freq (Hz)	N
Female Song Type 1: SR28											
2003	2.87±0.18	0.05±0.01	16.35±1.53	8.47±1.42	0.10±0.01	1163±37	2742±163	1436±208	3225±141	1074±39	6
2007	2.61±0.22	0.04±0.01	14.10±1.87	7.94±1.74	0.12±0.01	1200±45	2450±200	1461±255	2944±173	1132±48	4
2013	2.21±0.11	0.05±0.01	14.56±0.94	11.57±0.87	0.08±0.01	1098±22	2399±100	1228±127	3145±87	1034±24	16
F	5.61	0.37	0.61	2.83	2.69	2.61	1.63	0.57	0.82	1.77	-
p	**0.01**	0.69	0.55	0.09	0.08	0.10	0.22	0.58	0.45	0.19	-
Female Song Type 2: SR9											
2003	1.96±0.09	0.05±0.01	11.42±0.63	10.92±0.70	0.010±0.01	904±36	2483±103	1736±102	2989±85	861±29	8
2007	1.84±0.10	0.04±0.01	9.57±0.68	8.26±0.75	0.12±0.01	1103±39	2340±110	1408±109	2724±91	962±31	7
2013	1.74±0.07	0.04±0.01	11.52±0.48	11.27±0.53	0.09±0.01	1039±28	2583±78	1545±77	2925±64	937±22	14
F	1.83	2.40	3.04	**5.74**	**5.51**	7.51	1.64	2.41	2.41	3.35	-
p	0.18	0.11	0.07	**0.01**	**0.01**	0.003	0.21	0.10	0.10	0.05	-
Female Song Type 3: SR4											
2003	1.76±0.10	0.09±0.03	13.96±1.22	13.53±0.96	0.09±0.01	980±42	2180±145	891±180	2585±135	893±38	8
2007	1.70±0.10	0.05±0.03	12.62±1.30	14.47±1.03	0.08±0.01	926±45	1862±155	865±193	2131±1434	839±41	7
2013	1.77±0.103	0.09±0.03	14.14±1.30	16.17±1.03	0.08±0.01	929±45	1656±155	769±193	2049±144	850±41	7
F	0.15	0.68	0.42	1.77	0.33	0.48	3.13	0.11	4.39	0.54	-
p	0.86	0.52	0.67	0.20	0.72	0.62	0.07	0.89	**0.03**	0.59	-

Table 5.4: Females exhibited greater temporal variation than males in the 10 fine-scale structural measurements of two song types found in the repertoires of both males and females. Song types 2 and 3 are shown in Figure 5.1. Male CV and Female CV are the coefficient of variation (%) for each of the variables. Song length is the total length of the song, Term Length is the length of the terminal syllable; # Syllables is the total number of syllables in a song; Trill rate is the number of syllableless in the trill per second; Pause Length is the total duration of pauses in the song; Dom Freq of Trill is the dominant frequency that the trill is produced at; Term Syll BW is the bandwidth that the terminal syllable covers; Max Freq is the maximum frequency of the song; Min Freq is the minimum frequency of the song. Z indicates the z-scores calculated for the Fligner-Killeen test of coefficient of variation. All tests are significant at $p<0.05$.

	Song Type 2					*Song Type 3*				
Variable	**Male CV**	**Female CV**	**Z**	**p**	**Variable Sex**	**Male CV**	**Female CV**	**Z**	**p**	**Variable Sex**
Song Length (s)	10.49	14.99	1.55	0.12	-	10.53	14.15	1.08	0.28	-
Length of Term (s)	32.51	104.80	2.03	**0.04**	*female*	17.72	42.61	2.45	**0.01**	*female*
# Syllables	15.09	24.59	2.60	**<0.001**	*female*	12.43	17.06	2.12	**0.03**	*female*
Trill Rate (syllables/s)	11.56	19.26	0.93	0.35	-	11.72	21.88	2.84	**0.004**	*female*
Pause Length (s)	13.57	21.89	3.69	**<0.001**	*female*	14.48	25.73	3.25	**0.001**	*female*
Dom Freq of Trill (Hz)	4.21	12.29	5.07	**<0.001**	*female*	6.96	12.31	3.73	**<0.001**	*female*
Dom Freq of Term Syll (Hz)	18.35	23.49	1.61	0.11	-	15.71	11.73	-2.02	**0.04**	*male*
Bandwidth of Term Syll (Hz)	34.32	57.94	2.19	**0.03**	*female*	29.11	19.78	-2.42	**0.01**	*male*
Max freq (Hz)	14.31	19.29	1.10	0.27	-	17.07	8.72	-3.75	**<0.001**	*male*
Min freq (Hz)	5.29	12.25	2.86	**0.004**	*female*	6.12	9.46	2.77	**0.005**	*female*

Table 5.5: Measures of annual genetic diversity overall and for each sex. Genetic Diversity indices include the number of individuals genotyped / year (N), inbreeding coefficient (F_{IS}), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), the number of individuals assigned to a population outside of Santa Rosa using GENECLASS 2.0 (# Excluded) and Assignment index (A_I) based on 10 microsatellite loci.

							Overall		Males					Females				
Year	***N***	***F_{IS}***	***A_R***	***H_O***	***H_E***	***N_e***	***95% CI***	***# Excluded***	***A_I***	***F_{IS}***	***A_R***	***H_O***	***H_E***	***A_I***	***F_{IS}***	***A_R***	***H_O***	***H_E***
2003	26	0.046	7.74	0.661	0.679	607	95-∞	2	-0.22	0.033	6.61	0.680	0.678	0.35	0.096	5.41	0.630	0.659
2004	18	0.078	8.25	0.637	0.669	∞	110-∞	2	-0.11	0.072	6.76	0.660	0.674	0.21	0.116	5.60	0.600	0.624
2005	22	0.050	7.64	0.647	0.665	232	64-∞	2	-0.29	0.043	6.37	0.663	0.668	0.77	0.065	5.10	0.614	0.607
2006	33	0.056	7.78	0.628	0.654	212	85-∞	4	-0.13	0.094	6.35	0.595	0.638	0.23	0.008	5.43	0.677	0.656
2007	30	0.055	7.47	0.618	0.643	171	68-∞	4	0.24	0.053	5.95	0.614	0.629	-0.35	0.058	5.39	0.625	0.634
2008	42	0.078	7.39	0.623	0.655	197	96-3703	3	0.30	0.079	5.79	0.599	0.637	-0.49	0.019	5.52	0.663	0.654
2009	49	0.084	7.25	0.593	0.641	207	104-1956	2	0.57	0.111	5.74	0.568	0.627	-0.97	0.040	5.56	0.636	0.643
2010	42	0.061	7.25	0.592	0.622	289	110-∞	3	0.34	0.061	5.78	0.592	0.618	-0.67	0.055	5.30	0.594	0.604
2011	54	0.095	7.28	0.567	0.619	237	114-6334	1	-0.09	0.10	6.01	0.577	0.630	0.12	0.084	5.10	0.551	0.586
2012	71	0.111	7.61	0.575	0.642	282	152-1267	2	0.21	0.128	6.08	0.566	0.640	-0.41	0.071	5.40	0.593	0.624
2013	60	0.174	7.39	0.531	0.636	636	174-∞	2	0.34	0.204	5.81	0.507	0.625	-0.55	0.120	5.38	0.571	0.631

Table 5.6: Summary of Mantel and partial Mantel tests examining the relationship (coefficient= Mantel's *r*) between cultural distance, genetic distance, and temporal distance for both male and female Rufous-and-white Wrens. For genetic distance, we used the mean Nei's genetic distance for males and females combined. Periods are used to distinguish the variable that was controlled for in the partial Mantel tests. Bold values denote all tests that produced significant results.

Hypothesis tested	Male		Females	
	r	*p*	*r*	*p*
Cultural Distance x Genetic Distance	**0.79**	**<0.001**	**0.70**	**<0.001**
Cultural Distance x Temporal Distance	**0.89**	**<0.001**	**0.92**	**<0.001**
Genetic Distance x Temporal Distance	**0.79**	**<0.001**	**0.79**	**<0.001**
Cultural Distance x Genetic Distance|Temporal Distance	0.33	0.75	-0.09	0.82
Cultural Distance x Temporal Distance|Genetic Distance	**0.70**	**0.001**	**0.83**	**<0.001**
Genetic Distance x Temporal Distance|Cultural Distance	**0.29**	**0.04**	**0.51**	**0.003**

Figures

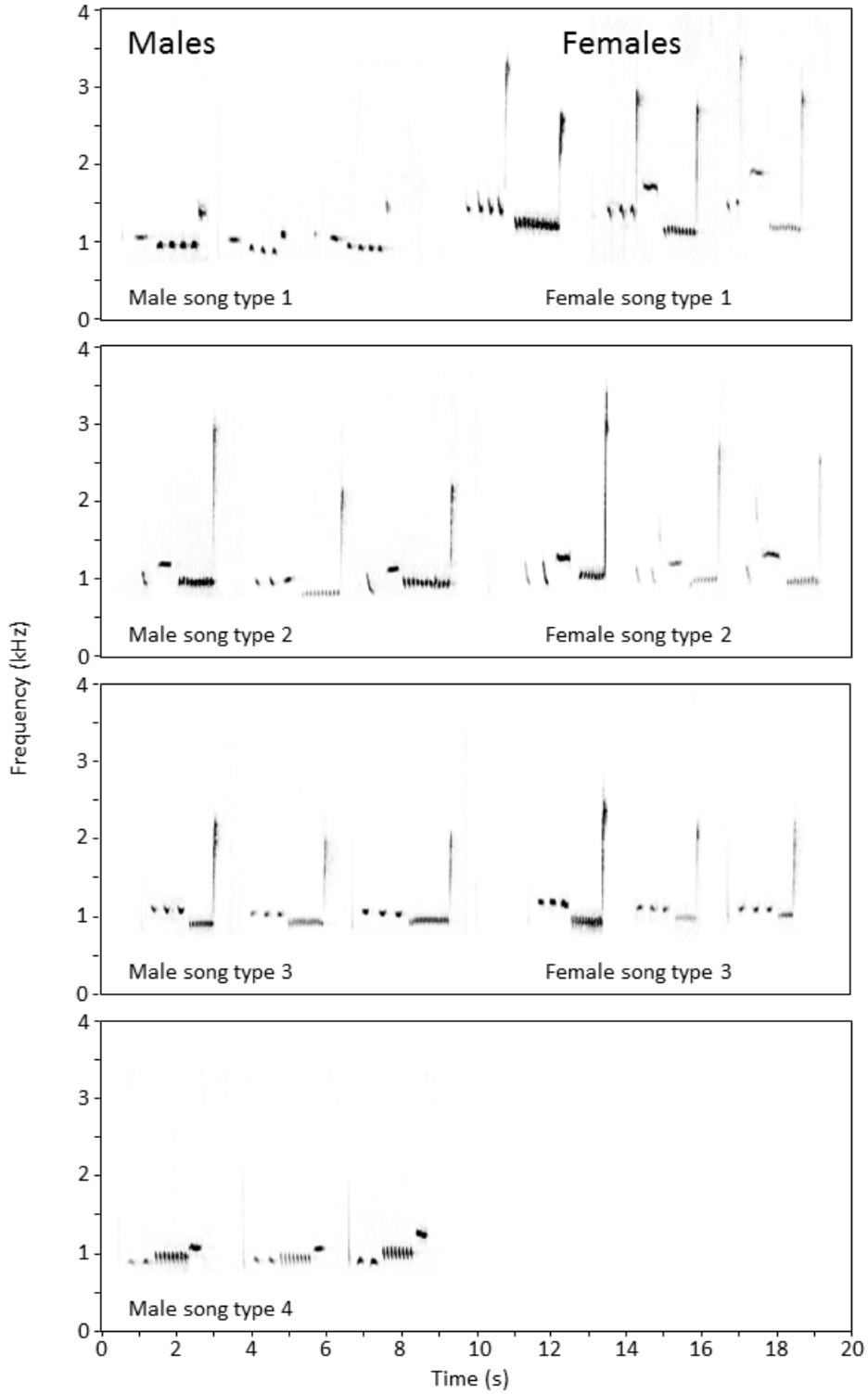

Figure 5.1: Sound spectrograms of the four male song types and three female song types recorded from 2003 (left), 2007 (center), and 2013 (right). Song types 2 and 3 are examples of song types found in the repertoires of both male and female Rufous-and-white Wrens.

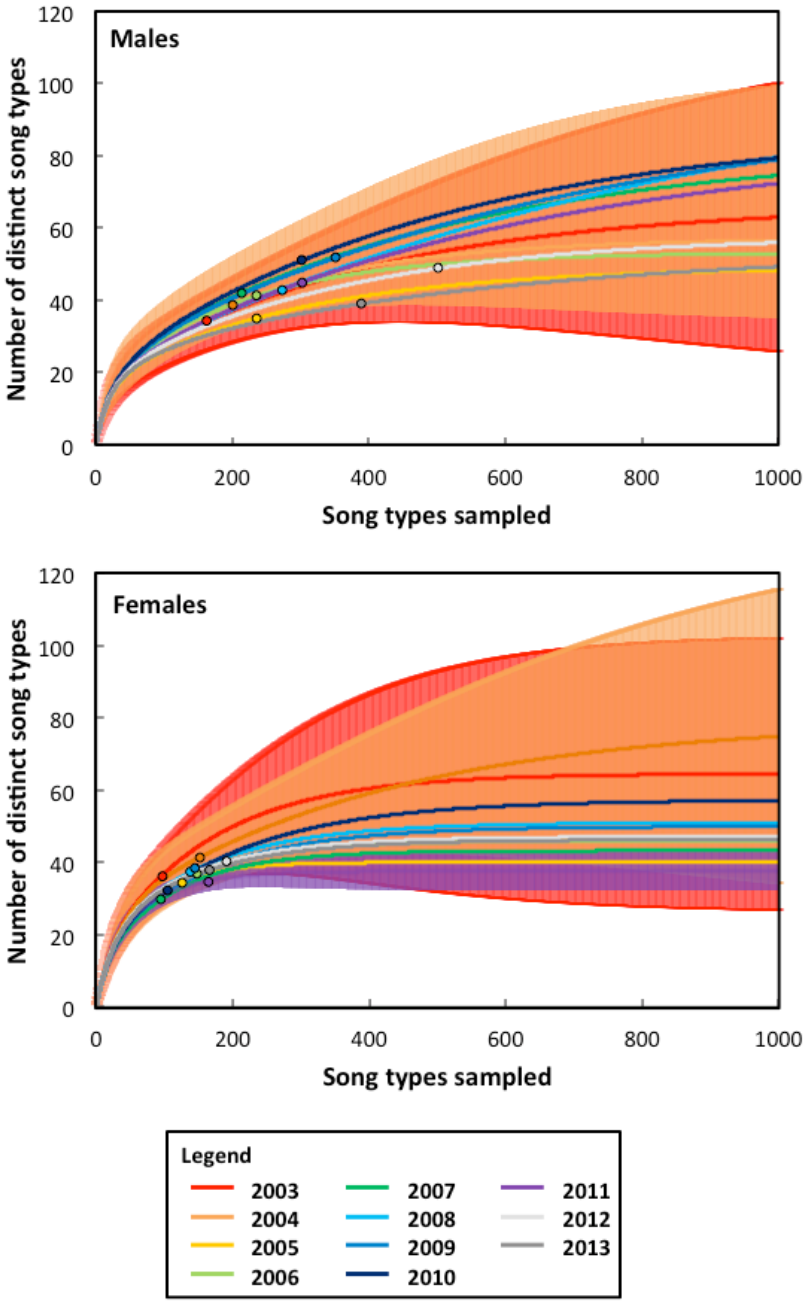

Figure 5.2: Rarefaction-extrapolation curves (based on 1000 randomizations without replacement) used to assess song-type richness across years for males (top) and females (bottom). Filled circles represent the number of song types sampled. Shaded polygons represent the 95% confidence intervals for each line. For both males and females 95% confidence intervals overlap across years demonstrating that song-type richness is equivalent across years indicating that changes in song type-frequencies for both sexes are not associated with increases or decreases in song type richness. For clarity, we only show two 95% confidence intervals for males and three 95% confidence intervals for females.

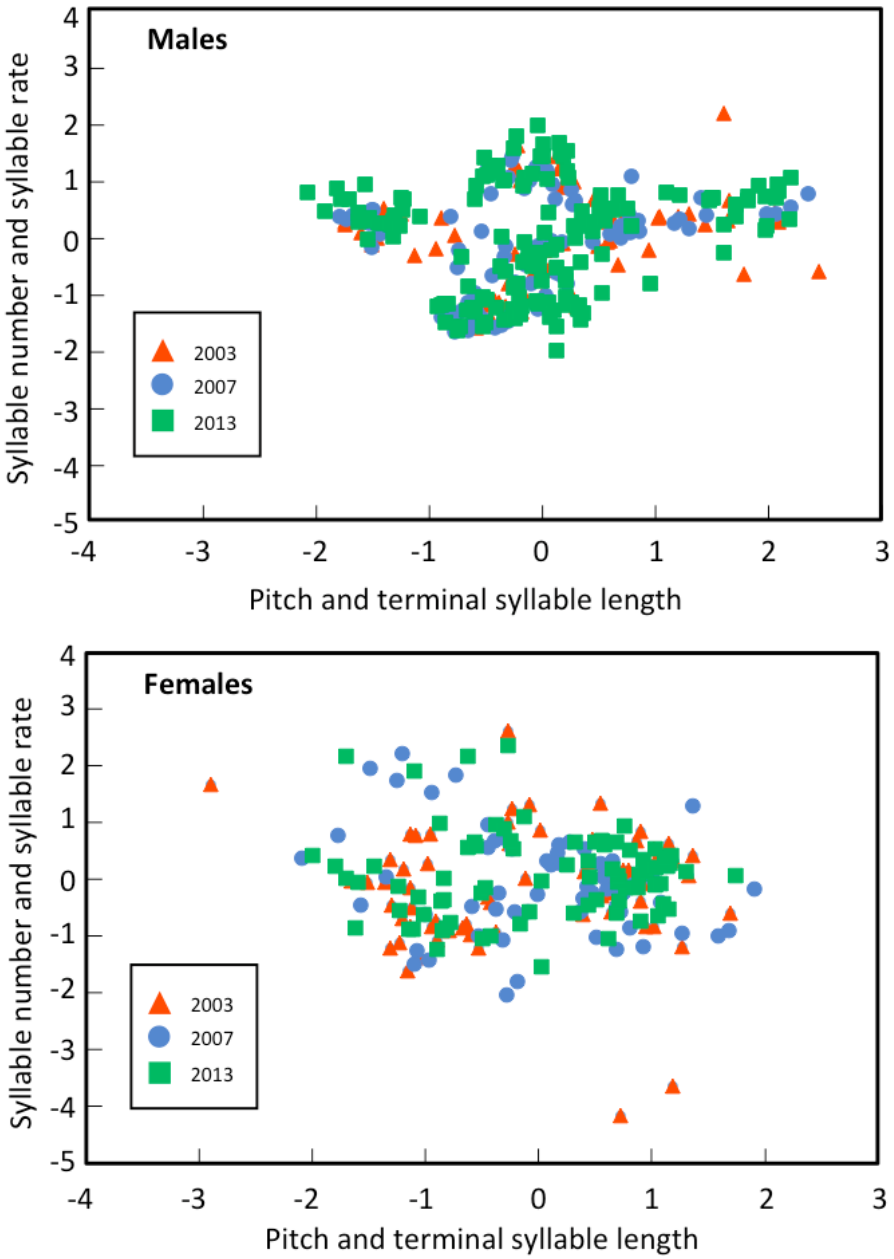

Figure 5.3: Principal component analysis of male (top) and female (bottom) Rufous-and-white Wren song types showed no temporal clustering based on 10 fine-structural measurements, demonstrating that overall the acoustic properties of songs are similar across a 10-year time period. Triangles represent songs from 2003, circles represent songs from 2007, and squares represent songs from 2013. The three temporal points were chosen based on genetic analyses that found 2003 and 2013 to be significantly different between from each other. 2007 represents a temporal period that was not significantly different from either 2003 or 2013. Plotted are the first principal component (representing pitch and terminal syllable length) and second principle component (representing syllable rate and syllable numbers).

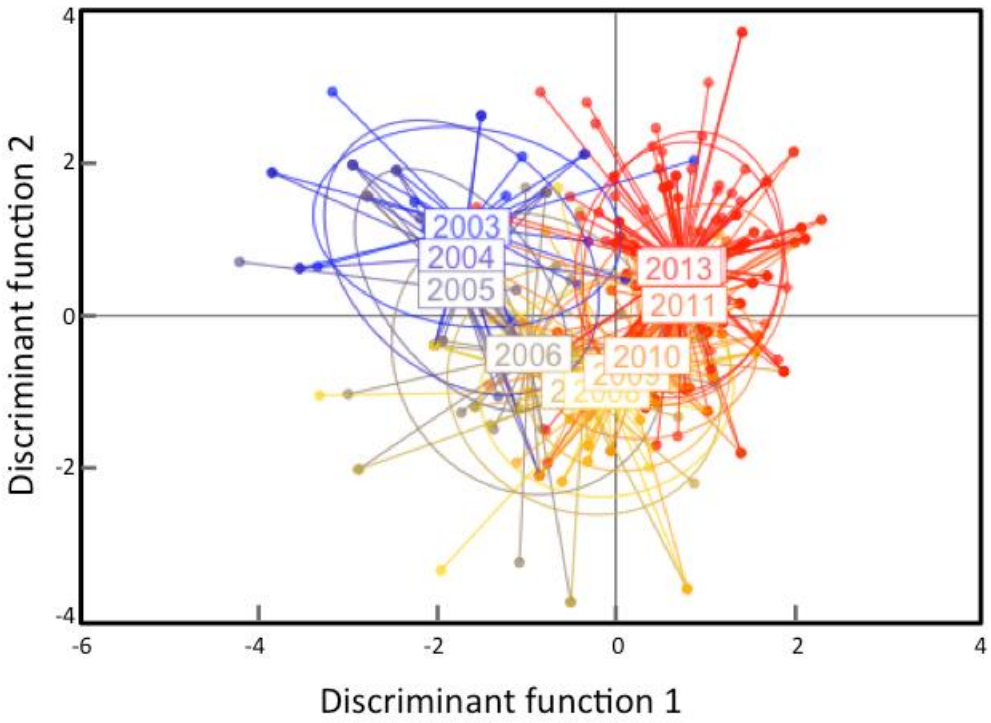

Figure 5.4: Discriminant analysis of principle components showing how closely years cluster together based on allele frequencies. Circles represent inertia ellipses, and lines link all points the center of each year. Colours represent year, with blue colours indicating the earliest years (starting in 2003) and red indicating the latest years (up to 2013). Earlier years (2003-2005) clustered closer together, while later years (2008-2013) formed the tightest clusters.

Supplementary information accompanying Chapter 5

Table 5.S1: Factor loadings for the first three principal components of fine scale acoustic measurements for male and female song types. Bolded values indicate variables with loadings greater than 0.3.

Variables	**Males**			**Females**		
	PC1	PC2	PC3	PC1	PC2	PC3
Song length (s)	-0.029	0.283	**0.710**	-0.105	**0.345**	**0.641**
Length of term (s)	**-0.747**	0.188	0.121	**-0.717**	0.019	0.285
# Syllables	0.074	**0.931**	0.186	-0.050	**0.857**	**0.335**
Trill rate (syllables/s)	-0.054	**0.971**	-0.168	0.038	**0.875**	-0.118
Pause length (s)	-0.154	**-0.853**	-0.035	-0.081	**-0.798**	0.119
Dom freq of trill (Hz)	0.201	-0.255	**0.740**	0.060	-0.099	**0.828**
Dom freq of term syll (Hz)	**0.803**	**0.343**	-0.164	**0.891**	0.103	0.031
Bandwidth of term syll (Hz)	**0.891**	0.144	0.017	**0.796**	0.058	0.281
Max freq (Hz)	0.186	-0.028	**-0.603**	0.272	-0.182	**0.599**
Min freq (Hz)	**0.917**	0.051	0.214	**0.844**	-0.037	0.231
Eigen value	3.86	2.12	1.53	3.25	2.43	1.35
% Variance explained	38.64	21.15	15.3	32.47	24.28	13.48
	Pitch and terminal syllable length	Syllable number and rate	Song length and frequency	Pitch and terminal syllable length	Syllable number and rate	Song length and frequency

Table 5.S2: Pairwise comparisons of male and female within-year song sharing. Values represent the mean difference in sharing between each year, with bolded values indicating significant differences in within-year song sharing following sequential Bonferroni corrections. Values below the diagonal are for males, while values above the diagonal are for females.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
2003		0.02	0.01	0.08	0.09	0.01	0.02	0.10	0.03	0.01	0.01
2004	0.01		0.03	0.10	0.11	0.03	0.01	**0.13**	0.01	0.02	0.03
2005	0.01	-0.01		0.07	0.08	0.01	0.02	0.10	0.03	0.01	0.01
2006	0.01	0.02	0.02		0.01	0.08	0.10	0.02	0.11	0.08	0.07
2007	0.03	0.04	0.04	0.02		0.08	0.10	0.01	0.12	0.09	0.08
2008	0.03	0.04	0.04	0.02	0.01		0.02	0.10	0.03	0.01	0.01
2009	0.09	0.10	**0.10**	0.08	0.06	0.06		0.12	0.01	0.01	0.03
2010	0.08	0.09	0.09	0.07	0.05	0.05	0.01		**0.13**	0.11	0.09
2011	0.04	0.05	0.05	0.03	0.01	0.01	0.05	0.04		0.02	0.04
2012	0.01	0.02	0.02	-0.01	0.02	0.02	**0.09**	0.07	0.03		0.01
2013	0.01	0.00	0.00	-0.02	0.04	0.04	**0.10**	**0.09**	0.05	0.02	

Table 5.S3: Pairwise comparisons measuring cultural distance. Distances were calculated by subtracting the Morisita overlap index of sharing from 1. Male values are shown below the diagonal, while female values are shown above the diagonal.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
2003	-	0.065	0.109	0.139	0.170	0.136	0.173	0.201	0.213	0.208	0.228
2004	0.022	-	0.041	0.055	0.114	0.082	0.119	0.134	0.169	0.172	0.187
2005	0.034	0.019	-	0.027	0.084	0.075	0.110	0.110	0.117	0.119	0.178
2006	0.041	0.032	0.027	-	0.039	0.054	0.088	0.100	0.145	0.150	0.211
2007	0.054	0.046	0.042	0.011	-	0.053	0.074	0.098	0.135	0.139	0.201
2008	0.063	0.058	0.051	0.027	0.017	-	0.034	0.052	0.098	0.104	0.144
2009	0.085	0.082	0.069	0.037	0.027	0.007	-	0.044	0.067	0.074	0.109
2010	0.075	0.068	0.060	0.033	0.024	0.012	0.008	-	0.062	0.074	0.099
2011	0.074	0.076	0.063	0.050	0.041	0.021	0.028	0.025	-	0.023	0.071
2012	0.073	0.088	0.078	0.063	0.056	0.036	0.040	0.032	0.011	-	0.055
2013	0.079	0.081	0.089	0.057	0.049	0.031	0.033	0.026	0.024	0.017	-

Chapter 6: Dispersal influences genetic and acoustic spatial structure in male and female tropical wrens*

*This work is the outcome of joint research with D. Heath, and D. Mennill

Chapter Summary

Animals display a wide variety of dispersal strategies, including sex-biased dispersal—a phenomenon commonly observed in both birds and mammals. Genetic structure and phenotypic structure are both influenced by dispersal, and these patterns may vary between the sexes because they exhibit dispersal differences and different life history traits. Here, we examined dispersal, spatial genetic structure, and spatial acoustic structure (i.e. population-wide patterns of song sharing) in Rufous-and-white Wrens, a year-round resident of Central and South America. Both sexes sing in this species, which allowed us to compare acoustic variation between sexes, and examine the relationship between dispersal and song sharing for each sex. Using a long-term dataset collected over an 11-year period, we used banding data and molecular genetic analysis to quantify natal and breeding dispersal distance in Rufous-and-white Wrens. Females dispersed farther from natal territories and dispersed more often between breeding territories than males. Natal dispersal appears to have greater influence than breeding dispersal on spatial genetic structure and spatial acoustic structure, given that the majority of breeding dispersal events resulted in individuals moving only short distances, into a neighbouring territory. Furthermore, analysis of genetic structure using spatial autocorrelation revealed that females showed no significant spatial genetic structure, whereas males showed significant spatial genetic structure, further supporting the idea that dispersal is female-biased. Spatial acoustic structure indicates that song sharing decreases with distance for both males and females, although males exhibited stronger spatial acoustic structure than females. Lastly, we measured the relationship between natal dispersal distance and song sharing. We found that sons shared fewer songs with their fathers the farther they dispersed from their natal territories, but that the proportion of songs daughters shared with their mothers was not significantly correlated with natal dispersal distance. Our results provide further insight into the

acoustic variation of male and female birds, and demonstrate that cultural differences between the sexes may correspond with sex-biased dispersal.

Introduction

Animals exhibit diverse dispersal strategies that may profoundly influence evolutionary trajectories (Clobert *et al*., 2009). These strategies vary both among and within species, including between-sex differences, as is common in many birds and mammals (Greenwood, 1980, Greenwood and Harvey, 1982). Females usually disperse farther than males in birds, whereas the reverse is true for mammals (Greenwood, 1980; Wolff, 1994; Clarke *et al.*, 1997). Dispersal is a critical component of the ecology, evolution, and spatial distribution of all animals, and has profound effects on the genetic and phenotypic structure of populations (Bohanuk, 1999; Ellers and Slabbekoorn, 2003; Clobert *et al*., 2009; Tarwater and Beissinger, 2012).

Phenotypic traits, such as acoustic signals, play a critical role in mate attraction and territory defense (Bradbury and Vehrencamp, 2011). Whereas most animals use innate vocalizations, other animals including some birds, bats, primates, elephants, seals, and cetaceans learn their vocalizations (Janik and Slater, 1997; Jarvis, 2004; Poole *et al*., 2005; Sanvito *et al*., 2007). In birds, vocal learning is common in three groups (songbirds, parrots, and hummingbirds; Jarvis, 2004). Studying learned vocalizations in relation to dispersal offers a unique opportunity to examine how animal movement shapes the evolution of acoustic signals (Wright and Wilkinson, 2001; Salinas-Melgoza and Wright, 2012). Depending on the timing of song learning, animals may introduce new songs into a population following dispersal (Lynch, 1996). These songs, however, will only become established if other birds learn them (Payne, 1996). Therefore, if there is strong selection for learning local songs by immigrants, dispersal may have little influence on the acoustic structure of a population (Beecher and Brenowitz, 2005).

Here we examine dispersal, spatial genetic structure, and spatial acoustic structure in male and female Rufous-and-white Wrens (*Thryophilus rufalbus*), a resident songbird found in Central America and northern South America (Stotz *et al*., 2007). In this species, both sexes sing repertoires

of up to 15 different song types, although males have significantly larger repertoires than females (Mennill and Vehrencamp, 2005; Harris *et al*., 2016). Many song types are sex-specific, although some songs are shared between males and females (Mennill and Vehrencamp, 2005). Song-learning has not been studied in this species, but our observations on acoustic similarity suggest that males learn songs primarily from males, and females learn songs from females, as has been demonstrated in other duetting species (Mennill and Rogers, 2006; Evans and Kleindorfer, 2016).

Female song is uncommon in north temperate systems, but it is widespread in the tropics (Slater and Mann, 2004), as well as being the ancestral trait in birds (Odom *et al*., 2014). Systems where both sexes sing are ideal for between-sex vocal comparisons, especially for learned traits like bird song, because dispersal can affect the transmission and variation of these signals (Pavalova *et al*., 2012). Given the prevalence of sex-biased dispersal in birds, comparisons of dispersal and acoustic variation between males and females offer the potential to further examine the relationship between dispersal and acoustic variation.

In this study, we quantify both natal dispersal distance and breeding dispersal distance in Rufous-and-white Wrens. We evaluate whether dispersal is sex-biased, and we compare natal dispersal distances with breeding dispersal distances to quantify and contrast juvenile dispersal and adult dispersal. We examine spatial genetic structure and spatial acoustic structure (i.e. population-wide patterns of song sharing) in both sexes to determine if there is a relationship between dispersal and song sharing. Previous investigations of within-sex song sharing in our study species have revealed that song sharing is lower for females than males (Mennill and Vehrencamp, 2005), and we sought to determine whether song sharing differences between sexes reflect dispersal differences between sexes. If one or both sexes show limited dispersal (i.e. if they disperse short distances), then song sharing should be correlated with distance and individuals should exhibit higher song sharing with neighbours, and lower song sharing with non-neighbours. By comparison, if

one or both sexes disperse greater distances, then song sharing should not be correlated with distance and patterns of song sharing are more likely to reflect patterns of selection (such as inter-sexual mating preferences or inter-sexual signaling strategies). Lastly, we compare the relationship between natal dispersal distance and song sharing between sons and fathers, and mothers and daughters, to examine whether males and females learn songs prior to dispersal and introduce their mother and father's songs into their eventual breeding neighbourhoods.

Methods

From 2003-2013 we monitored a population of Rufous-and white Wrens in Sector Santa Rosa of the Guanacaste Conservation Area in northwestern Costa Rica (10.85 °N, 85.60 °W; 286 m a.s.l.; Figure 6.1). We captured birds using mist nets and banded them with a unique band combination, consisting of one numbered aluminum band and three colour bands. We collected a small sample of blood (~100 μl) from the brachial vein, and stored blood samples in 95% ethanol or Queen's Lysis Buffer. Individuals were sexed based on the presence of a brood patch (females) and by singing behaviour (sexes can be distinguished based on fine structural differences in songs; Mennill and Vehrencamp 2005). Each year we identified all the birds in our study site, a 7 km-long patch of mature Neotropical dry forest, and collected data on their territory locations, breeding partners, and breeding activities. In addition to banding adult birds, we also banded nestlings when they were 7-12 days old, collecting small blood samples and providing each nestling with one numbered aluminum band and one colour band.

Estimating dispersal

We measured the natal dispersal distance and breeding dispersal distance of both male and female Rufous-and-white Wrens. We defined "natal dispersal" to be the movement from an individual's natal territory to their first breeding territory and we defined "breeding dispersal" to be the movement of a breeding adult from an established territory to another breeding territory

(Greenwood, 1980; Yáber and Rabenold, 2002). We considered the first breeding territory to be the territory where we observed an animal during its first breeding year. From 2003 to 2011 we banded 230 nestlings, and we used recapture/re-sight data to identify natal dispersal events. In total we re-sighted 21 individuals (9.1% of all banded juveniles) during the 11 years of our study. Nest depredation rates were extremely high at our population (up to 90%; Topp and Mennill, 2006) and the low percentage of recaptured birds likely reflects high rates of predation. In addition to the dispersal events we observed, other banded birds may disperse outside of the boundaries of our study population further contributing to the low percentage of recapture; concurrent genetic analysis of population structure shows that birds do sometimes disperse from our study site into nearby populations (Chapter 3).

Given the low recapture rates of juveniles over the 11 years, we used genetic analysis to identify parent-offspring dyads, and increase our pool of natal dispersers. We used 10 variable DNA microsatellites (see below) to identify potential parent-offspring dyads. Nestlings were banded in June and July of each year, but given the high nest predation rates these birds often re-nested following these events (Douglas *et al*., 2012), and could have bred until the end of August (Stiles and Skutch, 1989) greating an opportunity for dispersal events by unbanded individuals. At the beginning of each breeding season, approximately 1/3 of the adult birds were unbanded; some of these new birds may have been born in August in our population during the previous year. For this reason we used molecular genetic analysis to identify additional birds that were locally recruited.

To quantify breeding dispersal, we used only observational data (i.e. banding and re-sight data), and measured breeding dispersal distance following the same approach as our estimates of natal dispersal. We considered a breeding dispersal event to have occurred when if and individual was found in an alternate territory (in most cases with a different mate), either within the same breeding season or between consecutive breeding seasons.

We quantified natal dispersal and breeding dispersal using two different measurements. First we calculated straight-line distances between the center of a bird's natal territory and first breeding territory using the geographic distance calculator in GenALeX 6.5 (Peakall and Smouse, 2006; 2012). Second we measured dispersal distance as the number of breeding territories that an individual dispersed across (Cockburn *et al*., 2003; Sankamethawee *et al*., 2010).

We compared differences in natal and breeding dispersal distances between sexes using two-tailed t-tests. Given that we had fewer long-distance dispersal records (>1 km) than short-distance dispersal records (<1 km), our data violated normality and variance assumptions, and therefore we log-transformed natal dispersal distance data for both analyses (both straight-line distance and breeding territories). Additionally, we were interested in determining if adult males or adult females showed a greater propensity to disperse to another breeding territory (i.e. breeding dispersal). For this analysis we compared the number of male dispersers and non-dispersers to the number of female dispersers and non-dispersers using a Fisher's exact test in SPSS (version 23.0, SPSS Inc., Chicago, IL, USA).

Genetic analyses

We extracted DNA from blood samples using a Wizard Extraction Kit (Promega), and genotyped 213 individuals (123 males and 90 females) at 10 microsatellite loci. We used four previously designed microsatellite primer sets (*Th-Pl 14, Th-Pl 20, Th-Pl 30* (Brar *et al*., 2007), *RWWR 2c*: Hermann Mays personal communication), and developed six new microsatellite primer sets (*Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru 25;* Table 3.S1) following the microsatellite enrichment procedure detailed in Walter *et* al., (2007). All PCR reactions were conducted in 12.5 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 1.25 μL of 10x PCR buffer (Applied Biosystems), 0.5 μL of $MgCl_2$ (2.5 mM), 0.45 μl of dNTPs (0.2 mM), 0.05 μl of bovine serum albumin, and 0.5 U of Taq (Genscript, Applied Biosystems). For the primer sets *Tru 08, Tru 11, Tru 18, Tru 20, Tru 24, Tru*

25, and *RWWR 2c,* we included 1 μM each of the tel-forward, reverse, and M13 dye-labeled primer (GTAAAACGACGGCCAGT). For the remaining three primer sets (*Th-Pl 14, Th-Pl 20,* and *Th-Pl 30*) we used 1 μM each of the forward primer and the IR-dye labeled reverse primer. PCR conditions for *Th-Pl 14, Th-Pl 20,* and *Th-Pl 30* followed those described in Douglas *et al*. (2012), while for the remaining primer sets we used the following PCR conditions: one cycle of 94.0°C for 2 minutes, followed by 34 cycles of 94.0 °C for 10 seconds, 50.0°C for 10 seconds, 72.0°C for 30 seconds, followed by a final extension cycle of 72.0°C for 90 seconds, although for the primer set *Tru 24* we increased the annealing temperature (T_2) to 54.0°C to eliminate stuttering. PCR products were visualized on a 6% acrylamide gel on a Licor 4300 DNA analyzer. To ensure consistent sizing and scoring across gels, we ran controls with known size standards on each run. Allele sizes were scored using GeneImage IR 4.05 (Scanalytics, Inc., Rockville, MD).

The ten loci used in the analysis were polymorphic, ranging from low to high variability (average number of alleles per loci=12.3±2.78 alleles per locus; range 2-33 alleles). Mean observed heterozygosity was 0.59 ± 0.08, while the mean expected heterozygosity was 0.66 ± 0.09 across all 10 loci. Two loci (*Th-Pl 14* and *Th-Pl 30*) showed significant deviations from Hardy-Weinberg Equilibrium ($p<0.001$), and two of 45 loci combinations showed evidence of linkage disequilibrium following multiple corrections ($p<0.001$). Deviations from Hardy-Weinberg equilibrium indicate null alleles, but we used all 10 loci, and accounted for potential null alleles in our analysis (see below).

We calculated relatedness between individuals using software ML-Relate (Kalinowski*, et al*., 2006). ML-Relate uses a maximum-likelihood approach to estimate the probability that two individuals share an allele identical by descent at a given locus. Unlike other available software, ML-Relate can compensate for the presence of null alleles (Kalinowski*, et al*., 2006), giving a more accurate estimate of relatedness between individuals. The program classifies individuals into four different relationship categories: parent-offspring, full-siblings, half-siblings, and unrelated. Given

the goals of our study, we focused exclusively on identifying parent-offspring relationships. Given that null alleles can pose a problem in parentage analysis and potentially result in false parentage exclusions (Dakin and Avise, 2004), we tested for heterozygosity deficiency using the Monte-Carlo randomization test available in ML-Relate (Guo and Thompson, 1992). The program identified three loci (*Tru 08*, *Th-Pl 14*, and *Th-Pl 30*) with high probabilities of heterozygote excess ($p<0.001$), so we specified these three loci as having null alleles for our analysis. When null alleles are specified, the program estimates the frequency of null alleles following the methods of Kalinowski and Taper (2006).

To validate all parent-offspring dyads identified using ML-Relate, we used the specific "hypothesis testing" function in ML-Relate. This function tests the probability of a putative relationship (i.e. parent-offspring) versus an alternative relationship (i.e. unrelated). For this analysis we compared all parent-offspring relationships against full-sibling relationships, given that full-sibling relationships are most likely to be misidentified as parent-offspring relationships (Woltman *et* al., 2012). We tested all putative parent-offspring relationships by simulating 10 000 genotypes and only rejected the alternative hypothesis (full-sibling) if $p<0.05$. In all instances we identified the putative parent (i.e. father or mother) and offspring (i.e. son or daughter) using our banding data. We considered all birds banded first, temporally, to be the parent and the bird banded second to be the offspring (e.g. 2007 versus 2008). Additionally, we incorporated breeding data to help us correctly identify true parent-offspring relationships. For example, if the program failed to reject the alternative hypothesis (full-sibling) for two-males, we compared the putative offspring's genotype to the putative father's female partner from the previous breeding season. If a bird did not match for both parents, we considered this to be a Type I error (i.e. individuals that are not related but shared alleles across all loci by chance; Christie, 2010). The rate of extra-pair copulations and paternity is

low in this species (2% of all nestlings and 6% of all nests; Douglas *et al*., 2012), so it seems unlikely that a high proportion of mismatches with putative fathers would be due to extra-pair paternity.

Song analysis

We recorded the songs of individuals during the breeding season, in April through July of each year of the study, a time of year when vocal output is high for this species (Topp and Mennill, 2007). We recorded each individual on at least two separate occasions. The majority of our recordings were collected during focal recordings (60%), where we followed each bird around throughout its territory (each morning, from 0445h to 1100h) and confirmed the bird's identity during the recording. We recorded songs during focal recordings using a solid-state digital recorder (PMD-660 Marantz or PMD-661 Marantz; 44.1 KHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser MKH70 or ME67/K6). We supplemented focal recordings with recordings from automated recorders (see Harris *et al*., 2016 for details). We placed these recorders within the center of the territories of each focal pair, often within 10m of the focal pair's nest. We confirmed that the songs collected by these automated recorders were those of the intended pair by re-sighting the focal individuals in their territory after automated recording sessions, and by matching the songs collected by the automated recorders to the songs collected during focal recordings (Harris *et al.*, 2016).

Song type assignment and song sharing

We annotated all audio files using SYRINX-PC sound analysis software (J. Burt, Seattle, Washington, USA), and we built a library of all the song types in the repertoire of each male and female. To classify song types, we inspected the fine-structural characteristics of songs following the approach outlined in Harris *et al*. (2016). Previous work by Barker (2008) has shown that discriminant analysis can differentiate song types based on fine-structural measurements (i.e.

duration of song, maximum frequency, minimum frequency, and inter-syllable interval), and we incorporated these methods to help assign song types correctly.

For our analysis of song sharing, we focused exclusively on song sharing within each sex. While males and females share some song types, sharing between sexes is low (Mennill and Vehrencamp, 2005), suggesting that young males learn primarily from other males, while young females learn from other females. To measure song sharing, we calculated an adjusted Jaccard's coefficient (S_j) of sharing using the following formula (Tracy and Baker 1999):

$$S_j = c/((a+b+c)-d)$$

where a = the number of song types in individual A's repertoire but not individual B's, b = the number of song types in individual B's repertoire but not individual A's, c = the number of song types shared between two individuals, and d = the difference in repertoire size between individual A and B. We chose this coefficient because it accounts for differences in repertoire size (d) and birds in our population showed considerable variation in repertoire size (Harris *et al*., 2016).

Spatial genetic analysis

To examine patterns of fine-scale genetic structure and determine if Rufous-and-white Wrens exhibit sex-biased dispersal, we used spatial autocorrelation analysis (Smouse and Peakall, 1999). Spatial autocorrelation measures how closely correlated a variable is across geographic space. Previous work has shown that spatial autocorrelation is robust and capable of detecting patterns of sex-biased dispersal even when there are subtle differences in dispersal between sexes (Banks and Peakall, 2012). Unlike other spatial-analyses (e.g. Mantel tests) where raw geographic distances are compared, spatial-autocorrelation separates distances into classes. We used 1 km as our minimum geographical distance class for this analysis. We chose this value based on the distribution of individuals throughout our study site; the farthest gap between established territories in our study site is 1 km, and we feel that this is a biologically relevant distance for our

species, and this value is similar to distances used in other spatial genetic studies of resident bird populations (e.g. Liebgold *et al*., 2013). Distance classes were combined into four separate distance classes for our analysis (1 km, 2 km, 3 km, and 6 km). We combined all of the farthest distances into a single distance class 6 km; following the approach of Liebgold *et al*., 2013), because we had fewer samples at > 3 km, and combining them together gave us a larger sample size that was comparative to the sample sizes for our closest three distance classes.

For each distance class, GenAlEx calculates a coefficient of correlation (*r*), ranging between -1 and 1, to measure how similar, dissimilar, or random the genetic relationship among individuals is within distance classes. A significant positive value of *r* indicates that of individuals are more genetically similar than is expected by chance, while a negative significant *r* is indicative of individuals being less closely related than is expected by chance. When the value of *r* is not significantly different from zero, this indicates of random spatial distribution, where individuals are just as likely to be situated next to closely related individuals as they are next to unrelated individuals. In addition to calculating *r*, spatial-autocorrelation in GenAleX uses bootstrapping methods to generate upper and lower 95% confidence intervals around *r* (Peakall *et al.*, 2003).

We compared overall patterns of spatial genetic structure and patterns of spatial genetic structure between sexes using the "multiple populations analysis" in GenAlEx. This analysis combines data sets from multiple populations (in this case males and females) to produce a single correlogram that depicts the common spatial pattern across all populations. We generated separate genetic and geographic pairwise matrices for each sex; we used straight-line distance (km) between individuals as our measurement of geographic distance, and Nei's genetic distance as our measurement of genetic distance. We chose to analyze all individuals genotyped across the 11 years (123 males and 90 females) together rather than comparing patterns across years (Liebgold *et* al., 2013) because our sample sizes were uneven across years, ranging from 19-71 individuals across

years. Female sample sizes were especially low in some years (e.g. we had genetic data from only 6 individuals in 2004), and therefore we pooled all individuals together to improve our power of detecting patterns of fine-scale genetic structure and reduce the chances of error (Banks and Peakall, 2012). We ran the analysis for 999 permutations, following the protocol described by Peakall *et al.* (2003). We used Smouse and Peakall's test of heterogeneity (Smouse and Peakall, 2008) to determine if spatial genetic structure existed within each sex and overall (i.e. both sexes combined). This test uses an omega test (ω) to determine whether the correlogram exhibits significant spatial structure against the null hypothesis of no spatial genetic structure. We also compared spatial genetic structure between sexes to determine if males and females exhibited differences in spatial genetic structure. Similar to our overall analysis, we used Smouse and Peakall's test of heterogeneity to determine if spatial genetic structure patterns are different between each sex against the null hypothesis of no difference in spatial genetic structure between sexes (Smouse and Peakall, 2008). Tests of heterogeneity were considered significant only when $p<0.01$ (Smouse and Peakall, 2008). Lastly, we tested for heterogeneity between sexes within each distance class using the squared paired-sample t-test (t^2). This test allowed us to make direct comparisons within each distance class and determine if relatedness was significantly different between sexes.

Spatial acoustic analysis

In addition to analyzing fine-scale genetic structure, we also analyzed the spatial acoustic structure of males and females. For this analysis we wanted to know if males and females exhibit similar patterns of song sharing. While song sharing decreases as distance between breeding territories increases, both generally (Tracy and Baker, 1999; Podos and Warren, 2007) and in this species specifically (Mennill and Vehrencamp, 2005), we wanted to examine if spatial patterns of song sharing are comparable to spatial genetic structure patterns. If there are dispersal differences between the sexes, do patterns of song sharing reflect this? We conducted this analysis in GenAlEx,

using the multiple populations analysis with the same settings, and binned distance classes that we used in the genetic analysis. Similar to our genetic analysis we tested for heterogeneity overall, and also between sexes, using the previously described tests. Several other studies have employed spatial autocorrelation techniques in GenAlEx to analyse ecological and acoustic data (Peakall *et al.*, 2003; Pavalova *et al*., 2012), demonstrating the suitability of this technique in our study. To generate a pairwise distance matrix for acoustic dissimilarity, we converted our sharing coefficient (S_j) to a dissimilarity value by subtracting S_j from 1. Again, we created separate distance matrices for each sex; including all 237 colour banded individuals that we recorded full repertoires from in this analysis (134 males and 103 females).

Statistical analyses

We analyzed the relationship between song sharing and natal dispersal distance. Using all of the individuals identified as natal dispersers, we calculated the song sharing coefficient between all father-son pairs, and all mother-daughter pairs. For this analysis, we ran a multivariate linear regression model. We combined males and females together and used song sharing as our response variable and straight-line natal dispersal distance and sex as our fixed variables. We also analyzed sexes separately, but for this analysis we examined the relationship between song sharing and natal dispersal distance using a Pearson's correlation coefficient. We used this approach because our sample sizes were relatively small when the two sexes were analyzed separately. For both analyses, we used the log-transformed natal dispersal distance, as opposed to the raw distances, which violated assumptions of normality and variance. We excluded 2 males and 3 females because we did not have complete song repertoire data for the individual that dispersed. All statistical analyses were performed in SPSS (version 23.0, SPSS Inc., Chicago, IL, USA).

Results

Dispersal

Using both re-sight / recapture data and genetic analysis to identify parent-offspring dyads, we identified 26 natal dispersal events by male (n=11) and female (n=15) Rufous-and-white Wrens. We identified 21 natal dispersal events using re-sight / recapture data, and identified 19 natal dispersal events using microsatellite genotyping in ML-Relate. ML-Relate correctly rejected the null hypothesis (that animals were full-siblings and not parent-offspring) for all of the known 13 parent-offspring dyads identified by re-sight / recapture data that were included in our genetic analysis (genotyping data were missing for offspring or parents for 8 of the 21 natal dispersal events identified with re-sight / recapture data).

Our combined analysis of both sexes revealed that males and females dispersed 1230±57 m or 7.19±1.51 territories away from their natal territories. Between-sex comparisons suggest that natal dispersal is female-biased. Straight-line distance dispersed from natal territories was significantly different between the sexes (females: 1644±397 m, range= 121-4561 m; males: 675±190 m, range=113-2141 m; t=-1.73, p=0.05), and females dispersed farther from natal territories than males based on dispersal estimates using the number of territories an individual crossed (females: 9.67±2.37 territories, range=1-28; males: 3.82±0.88 territories, range=1-12; t=-1.77, p<0.05; Figure 6.2a). It is plausible that birds may have dispersed farther or outside of our population, given how low our recapture rates were and that we have detected gene flow between our study population and other nearby populations (Chapter 3).

Over the 11 years of our study, we observed 30 breeding dispersal events, with females dispersing from one breeding territory to another more often than males (17 of 103 females and 7 of 134 males dispersed from one breeding territory to another; χ^2 =8.14, p=0.005). Five individuals dispersed from breeding territories more than once: two females dispersed into a neighbouring

territory on two separate occasions, a third female dispersed from her breeding territory on three separate occasions, while two males dispersed into a neighbouring breeding territory, but eventually dispersed back to their original territory. Breeding dispersal distance estimates suggest that these movements were mostly local, given that males and females dispersed only 388±83 m or 2.17±0.50 territories (Figure 6.2b). Despite differences in the number of dispersal events between sexes, we only observed a borederline non-significant differences between sexes in straight-line breeding dispersal distance (females: 310±73 m, range: 100-1379 m; males: 572±214 m, range: 100-2200 m; t=1.64; p= 0.06) or the number of territories that an individual dispersed across (females: 1.95±0.5 territories, n=21, range: 1-10 territories; males: 2.67±1.21 territories, n=9, range: 1-12 territories; t=0.52; p=0.35).

Spatial genetic structure

Rufous-and-white Wrens exhibited significant spatial genetic structure (ω=31.81, p=0.001; Figure 6.3a; Table 6.1); individuals were more closely related to individuals at the closest distance class (1 km: *r*=0.007, p=0.001), but were less closely related to individuals at the two intermediate distance classes (2 km: *r*=-0.006, p=0.049; 3 km: *r*=-0.006, p=0.005). Males and females exhibited contrasting patterns of spatial genetic structure, and although these differences were not significant overall or between distance classes (ω=3.96, p=0.431; t^2=0.09-1.86, p>0.17), our results indicate that dispersal is female biased and that males exhibit greater philopatry. While spatial genetic structure was significant for males (ω=33.75, p=0.002; Figure 6.3b), female spatial genetic structure was not significant (ω=9.81, p=0.333; Figure 6.3c). Female genetic structure was not significant at any of the four distance classes (p>0.24). Males exhibited significant genetic structure at three of the four distance classes (1, 2, and 3 km); males were more closely related at the closest distance class (1 km: *r*=0.01, p=0.002), and were less closely related at the next two distance classes (2 km: *r*=-0.006, p=0.018; 3 km *r*=-0.006, p=0.018).

Spatial acoustic structure

Rufous-and-white Wrens exhibited significant spatial acoustic structure, thuse we reject the null hypothesis of no spatial acoustic structure (ω=43.28, p=0.001; Figure 4a; Table 6.1). Individuals shared more songs within the closest distance class (1 km: r=0.038, p=0.001) and shared fewer songs at the two farthest distance classes (3 km: *r*=-0.024, p=0.001; 6 km: r=-0.026, p=0.001; Figure 6.4a). Individually, males and females showed similar patterns of significant spatial acoustic structure (males: ω=43.13, p=0.001, Figure 6.4b; females: ω=31.78, p=0.001, Figure 6.4c), but spatial acoustic structure was significantly different between sexes (ω=18.58, p=0.001). Males exhibited greater song sharing than females at the closet distance class (1 km: males: *r*=0.058, p=0.001; females: *r*=0.013, p=0.001; t^2=28.99, p=0.001), but shared fewer songs than females at the two furthest distance classes (3 km: males: *r*=-0.032, p=0.002; females: *r*=-0.015, p=0.002; t^2=5.46, p=0.02; 6 km: *r*=-0.050, p=0.001; females: *r*=0.003, p=0.29; t^2=26.12, p=0.001). Overall, spatial acoustic patterns suggest that males share more songs with neighbours (i.e. birds <1 km away) than females, and that song sharing decreases with distance.

Correlation between song sharing and natal dispersal distance

Song sharing between sons and fathers was 0.59±0.05, while song sharing between daughters and mothers was 0.32±0.05. For our linear regression analysis of males and females combined, we found a statistically significant model ($F_{2,19}$= 8.16, adjusted R^2=0.42, p=0.003), showing that sex was a significant predictor of song sharing with the parent of the same sex (parameter estimate: -0.25±0.07, t=-3.46, p=0.003), and not dispersal distance (parameter estimate: -0.09±0.08, t=-1.13, p=0.27). When we analyzed sexes separately, however, we found that males and females demonstrated contrasting relationships between song sharing and dispersal distance: sons shared fewer songs with their fathers the farther they dispersed from their natal territory (r=-

0.74, p=0.02, n=9; Figure 6.5), whereas the number of songs a daughter shared with her mother was not correlated with natal dispersal distance(r=-0.01, p=0.99, n=12).

Discussion

We combined data direct observation and molecular data to quantify dispersal distances and dispersal patterns in a long-term study of Rufous-and-white Wrens. Our analysis of natal dispersal distance and spatial genetic structure indicate that dispersal is female-biased in Rufous-and-white Wrens. Female-biased dispersal is common in many bird species (Greenwood and Harvey, 1982; Clarke *et al*., 1997). In addition to demonstrating differences in spatial genetic structure between sexes, we also observed differences in the spatial acoustic structure of male and female Rufous-and-white Wrens. Males shared more songs with neighbours than birds further away, suggesting that song matching seems to be more important for males (Beecher *et al*., 2004). In contrast, females share fewer songs with their neighbours than males, suggesting that song matching is less important in females. Taken together, male and female song sharing patterns suggest that males learn more songs from breeding territory neighbours than females do. Below we discuss further how differences in behaviour may influence differences in male and female spatial acoustic structure.

Patterns of dispersal

Many tropical species occupy territories throughout the year (Greenberg and Gradwohl, 1986; 1997; Morton *et al*., 2000; Tobias *et al*., 2011), demonstrate high local recruitment (Gill and Stutchbury, 2006; Woltmann *et al*., 2012), and are thereby thought to exhibit limited dispersal (Moore *et al*., 2008; but see Van Houten, 2007). Although sex-biased dispersal has been more commonly studied in temperate species (Greenwood and Harvey, 1982; Clark *et al.*, 1997; Liebgold *et al*., 2013), our study further demonstrates that tropical species also display sex-biased dispersal as has been shown in other tropical birds (Yáber and Rabenold, 2002; Williams and Rabenold, 2005;

Berg *et al*., 2009; Sankamethawee *et al*., 2010; Pavlova *et al*., 2012; Ribeiro *et al*., 2012; Vangestel *et al*., 2013). Our direct measurements of natal dispersal distances are comparable to those observed in other tropical bird species, providing further insight into the movement of young animals living at tropical latitudes (e.g. Martín and Bucher, 1993; Woodworth *et al*., 1998; Woltmann *et al*., 2012). It is important to note that our estimates of dispersal may be conservative, especially since our analysis is biased towards individuals that settled in our population; it is conceivable that individuals dispersed farther and settled into territories outside of our study population. Although breeding dispersal is commonly observed in some species (Ribeiro *et* al., 2012), our estimates of breeding dispersal suggest that breeding dispersal is female-biased, infrequent, and occurs over relatively short distances (Mulder, 1995; Woodworth *et al*., 1998; Yáber and Rabenold, 2002). These patterns indicate that natal dispersal has a greater influence than breeding dispersal on spatial acoustic structure and spatial genetic structure (Newton, 2007).

Similar to other resident species, in both the North Temperate Zone and the Tropics, we detected stronger spatial genetic structure for males than females in Rufous-and-white Wrens (Yáber and Rabenold, 2002; Sankamethawee *et al*., 2010; Ribero *et al*., 2012 Liebgold *et* al., 2013; Vangestel *et al*., 2013). The prevalence of female-biased dispersal at local scales matches dispersal patterns at broad scales, where using genetic analysis we have documented that females are the more dispersive sex in this species (Chapter 3). Overall, our results highlight that tropical species may not be as sedentary as previously thought (Stutchbury and Morton, 2001). In particular the dispersal capabilities of females add to the growing literature suggesting that tropical birds may be capable of moving farther distances than we have recognized historically (Van Houten *et* al., 2007). While our results indicate that males are more philopatric than females, it is noteworthy that dispersal patterns may vary between years. Whereas long-term patterns may indicate female-biased

dispersal, dispersal patterns may show no bias or even male bias in some years (as in Eikenaar *et al*., 2010; Richardson *et al*., 2010; Liebgold *et al*., 2013).

Spatial structure of songs

Dispersal influences spatial genetic structure, and dispersal also influences spatial acoustic structure (Pavlova *et al*., 2013; Fayet *et al*., 2014). In Rufous-and-white Wrens, males and females showed similar spatial acoustic structure, sharing more songs with their nearest neighbours, but males exhibited stronger spatial acoustic structure than females (Mennill and Vehrencamp, 2005). Generally, studies of duetting species have shown that males exhibit higher song sharing and syllable sharing than females (Brown and Farbaugh, 1997; Mennill and Vehrencamp, 2005; Hall *et* al., 2015), although there are exceptions (e.g. Colombelli-Négrel, 2016). Between-sex differences in song sharing may reflect differences in the way that male and female birds use their songs and repertoires. For example male Bay Wrens (*Cantorchilus nigricapillus*) use their songs to communicate with both males and females: male songs are used to attract females when males are unpaired and males use their songs to acoustically guard mates from rival males when paired. By comparison female Bay Wrens do not appear to use their songs to attract mates, but instead use their songs to defend territories against conspecific females (Levin, 1996a; Levin, 1996b).

During territorial displays male birds often match songs with neighbours (reviewed in King and MacGregor, 2016), and therefore neighbouring individuals usually share a high proportion of songs or song types with their neighbours (Nelson, 2000; Beecher *et al*., 2000; Trillo and Vehrencamp, 2005). Sharing more songs with territorial neighbours may bestow several advantages, including increased reproductive success, and increased territory occupancy than birds that share-fewer songs with neighbours (Payne and Payne, 1997; Beecher *et al.*, 2000; Beecher and Brenowitz, 2005). Additionally song sharing may reflect physiological condition and population of origin (Stewart and MacDougall-Shackleton, 2008). While song type matching is common in males there

are fewer examples of it in females (see Marshall-Ball and Slater, 2004; Marshall-Ball *et al*., 2006). Similar to male song, female song is a multifunctional signal, and while some females birds use their songs to defend territories and mates (Levin, 1996b; Logue, 2007; Tobias and Seddon, 2009; Templeton *et al*., 2011; Illes, 2014; Cain and Langmore, 2015), others use their songs primarily for communicating with their breeding partners (i.e locating them in densely vegetated habitats) or coordinating breeding activities (i.e nest building; Mays *et* al., 2006; Mennill and Vehrencamp, 2008; Templeton *et al*., 2013a; Hall *et al*., 2015). In duetting species, repertoires may serve different functions, including territory defence or mate guarding (Hall, 2004). Therefore, matching song types or phrases with mates may be more important than matching conspecifics in duetting species (Marshall-Ball *et al*., 2004; Logue, 2007), especially since some duetting species adhere to duet codes (where males and females answer each others songs with distinct song types; Logue, 2006; Templeton *et al*., 2013b).

Between-sex differences in song sharing may also reflect sex-specific tutor differences. Evans and Kleindorfer (2016) found that Superb Fairy-wren (*Malarus cyaneus*) sons and daughters learn song elements from both their social fathers and mothers. Studies of two temperate songbirds, however, suggest that young males learn the majority of their songs from natal and breeding territory neighbours (Wheelwright *et al*., 2008; Nelson and Poesel, 2014). In our study we observed that sons sing fewer songs from their fathers the farther they disperse from their natal territories. By comparison, the number of songs that daughters learn from their mothers was not correlated with natal dispersal distance. These results suggest that males learn songs post-dispersal, and primarily from breeding territorial neighbours (Payne, 1981; Wright *et* al., 2005). In contrast, female song-learning patterns are less clear, although spatial acoustic structure suggests that repertories are more similar between neighbours, suggesting that similar patterns of post-dispersal learning may apply to females also. Lower rates of song sharing and weaker spatial acoustic

structure observed in females may be a byproduct of dispersal differences between sexes. For example, males appear to move to the first available breeding territory and are thereby exposed to a limited number of potential song tutors (on average males dispersed only four territories away from their natal territories). In contrast, due to the greater dispersal distances of females, young females may move around more looking for potential mates and therefore encounter more song tutors, either through their own movements, or by the movements of other females (i.e. females dispersed almost ten territories away from natal territories, suggesting that there are more females moving around and singing while they assess potential mates), thus resulting in lower patterns of spatial acoustic structure. Alternatively if dispersal is delayed in females (as is observed in some tropical species; Russell, 2000; Russell *et al*., 2004; Gill and Stutchbury, 2010; Tarwater and Brawn, 2010), individuals may learn more songs from their mothers or natal territory neighbours, thereby explaining the non-significant relationship observed between natal dispersal and the proportion of songs shared between mothers and daughters.

Across species where females sing, males and females not only vary in their vocal output, but also in how they use their songs. Differences in acoustic variation may reflect selection differences between sexes (Mennill and Rogers, 2006, Tobias *et al*., 2011; Hall *et al*., 2015) but they may also reflect developmental or song-learning differences between sexes (Beecher and Brenowitz, 2005). For example, comparative studies have demonstrated that the song-control regions of male songbirds are larger than the song-control regions of female songbirds; differences in song output are related to the volume of the song control region (MacDougall-Shackleton and Ball, 1999). Rufous-and-white Wrens also exhibit sexual dimorphism with respect the volume of the song control region, and these differences are correlated with repertoire size differences between sexes (Brenowitz and Arnold, 1986). Patterns of song ontogeny, and song-learning patterns of female birds

remain poorly understood in female songbirds (Riebel *et al.*, 2005), and therefore further research is necessary to understand how these differences affect acoustic structure.

Conclusion

Like many other vertebrate species, Rufous-and-white wrens display sex-biased dispersal. Males settle closer to natal territories than females, indicating that they likely disperse into the nearest available territory. By comparison females disperse farther from natal territories, suggesting that they do not move into the first available breeding territory. Our results reveal a relationship between dispersal and acoustic variation in a tropical songbird where both sexes sing. We found a strong correlation between the level of song sharing between fathers and sons and dispersal distance, whereas we found no relationship between dispersal distance and the level of song sharing between mothers and daughters. These results indicate that males learn songs from territorial neighbours, and this behaviour may be important if song matching plays an important in social interactions between males. Females share fewer songs with neighbours than males suggesting that vocal repertoire matching is less important for females. Additionally the lack of matching with neighbours could arise because females are learning songs throughout the dispersal process as they search for and assess potential breeding partners. Finally natal dispersal, but not breeding dispersal, appears to shape the spatial acoustic structure of males and females, given that breeding dispersal is infrequent and occurs over short distances. Taken together, our results provide greater insight into behavioural differences and cultural differences between male and female tropical birds.

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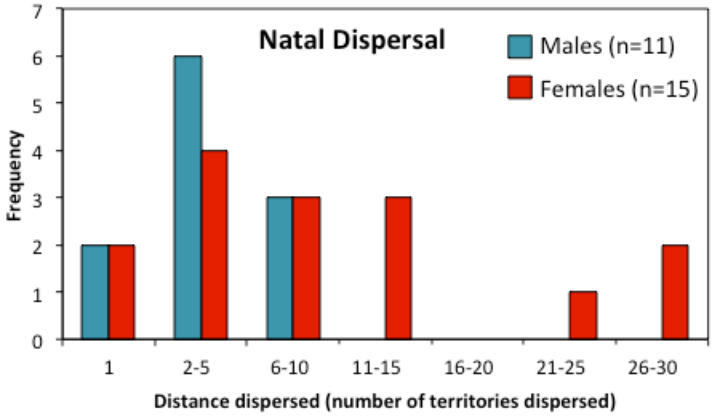
Tables

Table 6.1: Results of genetic and acoustic spatial autocorrelation of all birds combined and within each sex. Measurement indicates the sex and level (genetic vs. acoustic) measured. N equals the number of pairwise comparisons for a given distance class for the calculation of *r*; *r* equals the correlation for each distance class; 95% CI represents the confidence intervals for each distance class, *p* denotes the significance of tests for $r > 0$ if *r* is positive and $r < 0$ if *r* is negative (α=0.05); ω equals the test of heterogeneity value, p_ω=denotes the significance of heterogeneity test ((α=0.01).

Measurement	$N_{1\ km}$	$r_{1\ km}$	95% $CI_{1\ km}$	$p_{1\ km}$	$N_{2\ km}$	$r_{2\ km}$	95% $CI_{2\ km}$	$p_{2\ km}$	N1	$r_{3\ km}$	95% $CI_{3\ km}$	$p_{3\ km}$	$N_{6\ km}$	$r_{6\ km}$	95% $CI_{6\ km}$	$P_{6\ km}$	ω	p_ω
All birds Genetic	**3409**	**0.007**	**0.004 to -0.004**	**0.001**	**2867**	**-0.003**	**0.004 to -0.004**	**0.049**	**2736**	**-0.005**	**0.004 to -0.004**	**0.005**	2496	0.000	0.004 to -0.005	0.504	**31.81**	**0.001**
Males Genetic	**2217**	**0.010**	**0.006 to -0.004**	**0.002**	**1881**	**-0.006**	**0.005 to -0.005**	**0.018**	**1924**	**-0.006**	**0.005 to -0.005**	**0.012**	1481	0.001	0.005 to -0.006	0.363	**33.75**	**0.002**
Females Genetic	1192	0.001	0.007 to -0.006	0.338	986	0.002	0.006 to -0.007	0.322	812	-0.003	0.007 to -0.008	0.227	1015	-0.001	0.006 to -0.007	0.367	9.31	0.333
All birds acoustic	**4141**	**0.04**	**0.006 to -0.006**	**0.001**	3802	0.001	0.005 to -0.006	0.400	**3437**	**-0.024**	**0.005 to -0.007**	**0.001**	**2784**	**-0.026**	**-0.006 to 0.006**	**0.001**	**43.05**	**0.001**
Males Acoustic	**2578**	**0.058**	**0.009 to -0.007**	**0.001**	2388	0.003	0.008 to -0.009	0.201	**2190**	**-0.032**	**0.008 to -0.010**	**0.001**	**1755**	**-0.050**	**0.008 to -0.009**	**0.001**	**43.13**	**0.001**
Females Acoustic	**1563**	**0.013**	**0.008 to -0.007**	**0.001**	1414	-0.003	0.007 to -0.007	0.235	**1247**	**-0.015**	**0.007 to -0.008**	**0.002**	1029	0.003	0.007 to -0.008	0.289	**31.78**	**0.001**

Figures

Figure 6.1: Map of study area, showing the distribution of the breeding areas of Rufous-and-white Wrens in sector Santa Rosa of the Guanacaste Conservation Area.



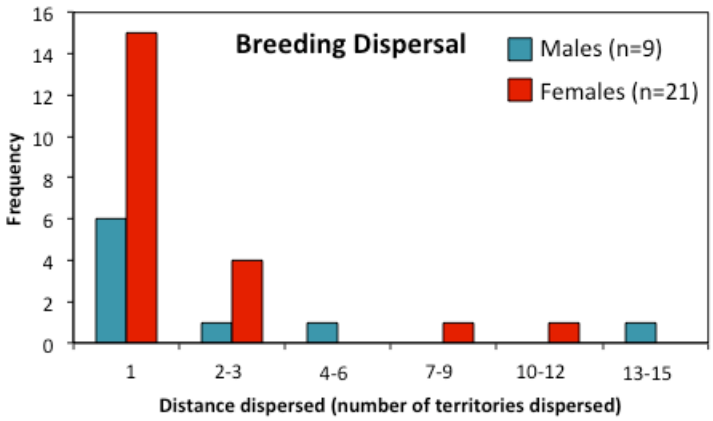


Figure 6.2: (a) Female and male natal dispersal of Rufous-and-white Wrens measured as the number of territories dispersed before establishing their first breeding territories. Overall males dispersed fewer territories from their natal territories than females. (b) Female and male breeding dispersal, measured as the number of territories individuals dispersed before establishing a new breeding territory. Overall males and females dispersed relatively short distances, given that 70% of individuals moved into an adjacent breeding territory.

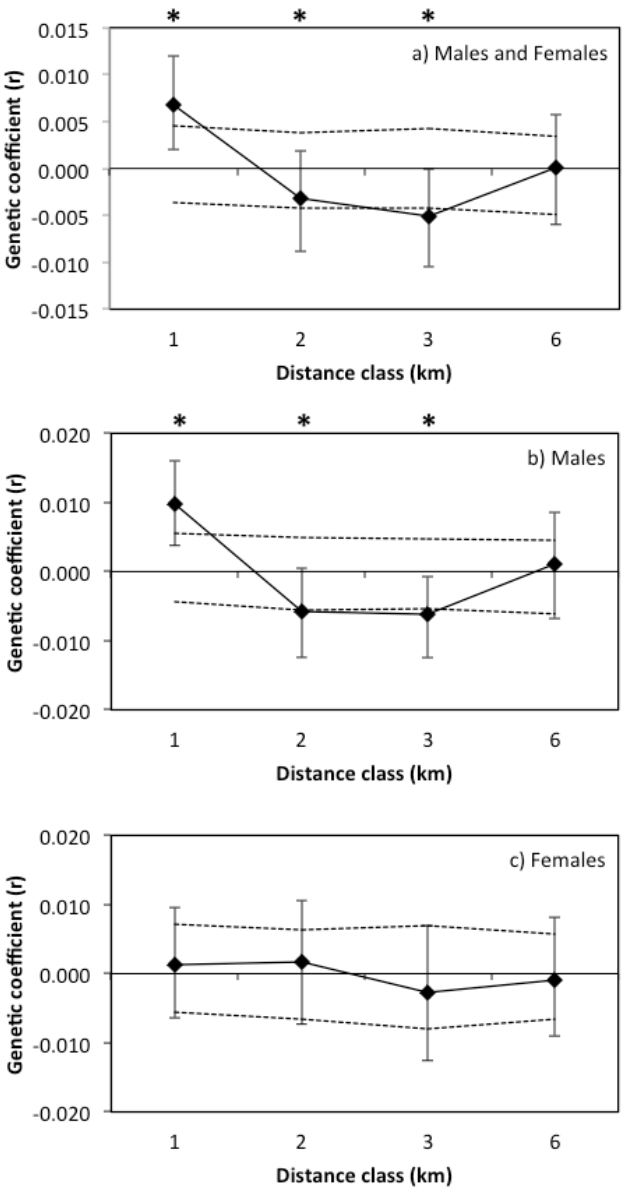


Figure 6.3: Correlograms showing the spatial genetic autocorrelation (*r*) with the designated distance classes for (a) males and females combined, (b) males only, and (c) females only. Male Rufous-and-white Wrens were more genetically similar at the closest distance class, but became more dissimilar as distance increased. By comparisons females exhibited no significant genetic structure at any of the four distance classes. Dashed black lines represent the 95% upper and lower confidence limits determined using bootstrapping. Asterisks denote the distance classes where song sharing was significantly higher or lower from what was expected by chance ($p<0.05$).

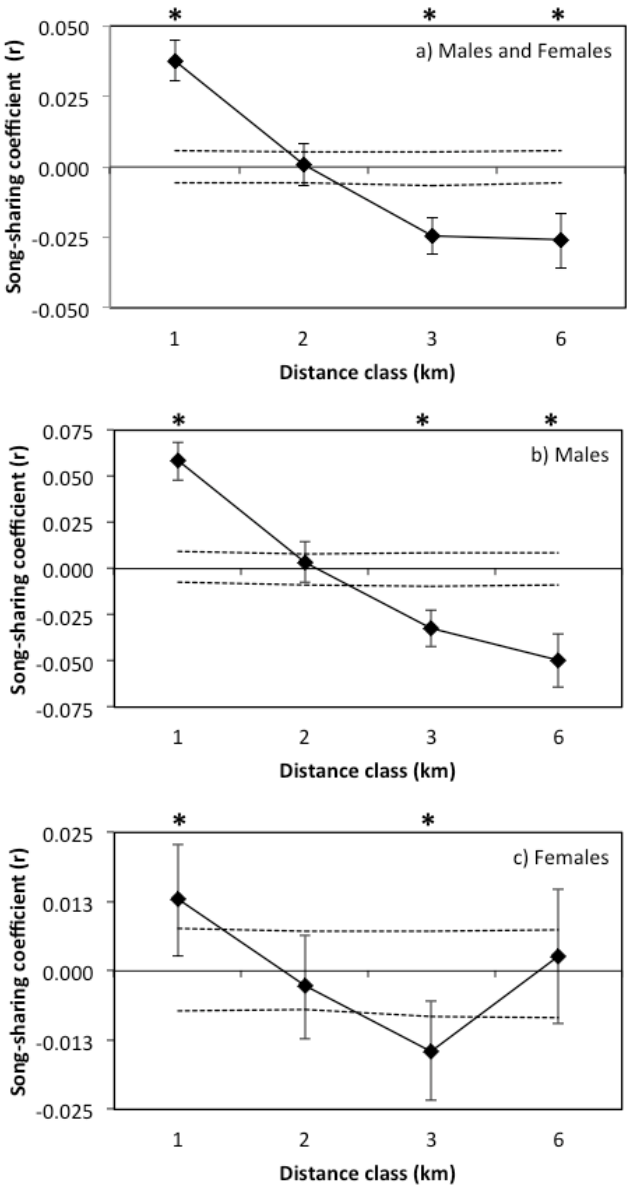


Figure 6.4: Correlograms showing the spatial acoustic autocorrelation (*r*) with the designated distance classes for (a) males and females combined, (b) males only, and (c) females only. Male and female Rufous-and-white Wrens had more similar repertoires at the closest distance class, but repertoires became more dissimilar as distance increased although for females repertoire sharing was not significantly different from random at the furthest distance class. Dashed red lines represent the 95% upper and lower confidence limits determined using bootstrapping. Asterisks denote the distance classes where song sharing was significantly higher or lower from what was expected by chance (α=0.05).

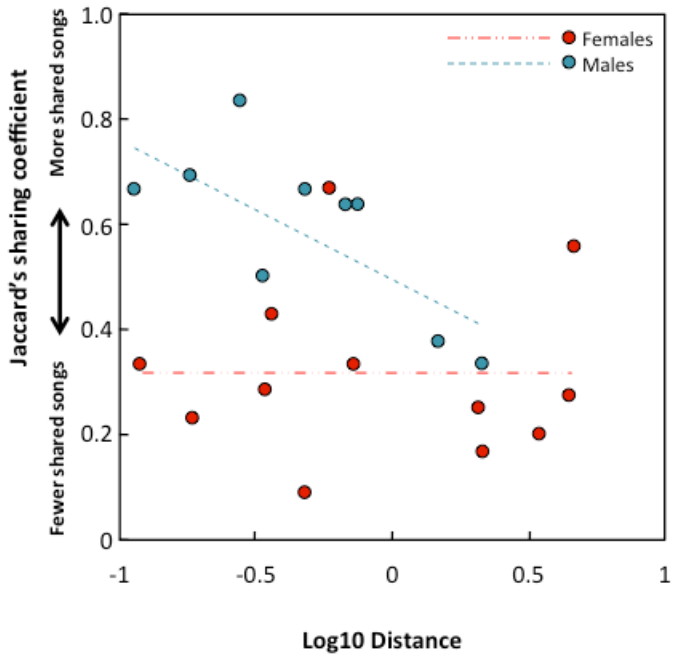


Figure 6.5: Song sharing (with the parent of the same sex) significantly decreases with natal dispersal distance in male (open circles) but not female (closed circles) Rufous-and-white Wrens. For our analysis of males and females together, dispersal distance did not significantly predict song sharing (t=-1.13, p=0.27). Dotted line shows the relationship for males (r=-0.74, p=0.02), while the dashed line shows the relationship for females (r=-0.01, p=0.99).

Chapter 7: General Discussion

The evolutionary forces that act on male song have been well studied; however, less is known about the evolutionary forces that act on female song (Riebel *et al*., 2005; Wilkins *et al*., 2013). Recent work indicates that female song is more common in birds than previously thought, and therefore further studies are necessary to determine whether male and female songs show similar patterns of evolution (Odom *et al*., 2014; Price, 2015). Given that males and females exhibit very different life history characteristics, there may also be differences in the way males and females use their songs, and therefore different evolutionary pressures may act on the acoustic properties of each sex (Price, 2015).

In my dissertation I analyzed acoustic variation in male and female Rufous-and-white Wrens (*Thryophilus rufalbus*) and compared patterns of acoustic variation between sexes. This body of work represents one of the few studies to compare acoustic variation between males and females, and test the evolutionary factors that drive song divergence between the sexes (see also Mennill and Rogers, 2006). I combined acoustic analyses with molecular genetic analyses, and a sound transmission study, to test the role that acoustic adaptation, dispersal, drift, immigration, and isolation have on the evolution of songs in male and female Rufous-and-white Wrens. My results provide insight into the evolution of male and female culture, and indicate that cultural drift is a significant driver of both male and female Rufous-and-white Wren songs (Chapter 3 and 5). Although ecological and environmental variables affect acoustic variation in some species (Handford and Lougheed, 1991; Slabbekoorn and Smith, 2002), my results suggest that Rufous-and-white Wren songs do not exhibit specific acoustic adaptations of different types of forest habitat, although environment does influence the transmission of male and female songs (Chapter 2).

My research provides greater insight into the process of vocal learning. Although I described significant dispersal between my study populations, first-generation migrants do not appear to introduce songs from their natal population into their breeding populations (Chapter 4), as has been

suggested in other species (Stewart and MacDougall-Shackleton, 2008; Fayet *et al*., 2014). My results instead suggest that first-generation migrants learn local songs following natal dispersal, and match their song repertoires with other animals in their breeding population, as predicted by the Social Adaptation Hypothesis (Payne, 1981).

My research also reveals that males and females exhibit dispersal differences; females disperse farther from natal territories than males, and these dispersal differences influence differences in spatial acoustic structure between sexes (Chapter 6). Natal dispersal influences both spatial genetic structure and spatial acoustic structure, whereas breeding dispersal was infrequent and mostly occurred over short distances, often resulting in individuals moving into a neighbouring territory. In this General Discussion of my dissertation I summarize the results of my dissertation and I discuss how the different evolutionary forces act together to influence male and female songs.

Do males and females exhibit similar patterns of acoustic variation?

My data chapters revealed that male and female Rufous-and-white Wrens show both cultural similarities and differences. Males and females exhibited similar patterns of acoustic divergence, inter-annual variation in acoustic structure, and changes in song-type frequencies over time (i.e changes in the distribution of songs being learned over time), and similar sound transmission properties. These results indicate that similar evolutionary forces act on both male and female songs. This research adds to the growing body of work that is changing long-standing assumptions about bird song and, in particular, female song (Price *et al*., 2009; Odom *et al*., 2014; Price, 2015).

With respect to cultural differences, I observed that female songs exhibit greater acoustic divergence among populations than male songs (based on pairwise Euclidean Distances; Chapter 3). Female songs appear to evolve faster than male songs, because female songs show greater inter-annual variation in the acoustic structure of songs than do male songs (Chapter 4). Additionally,

females exhibit lower song sharing and greater changes in song type frequencies than males (Chapter 4). These patterns may promote greater acoustic differentiation between populations for both sexes, but also indicate that there may be sex-based differences in selection pressures acting on songs. In particular, males and females appear to use their songs differently; song type matching appears to be important in males (Beecher *et al*., 2000), but less important in females (Hall *et al*., 2015). There is considerable evidence showing that song is a multifunctional signal in males used for territory defence and mate attraction (reviewed in Catchpole and Slater, 2008). The primary function of female song instead appears to be territory defence (Mennill, 2006; Hall *et al*., 2015), although some females do use their songs to deter female rivals (Iiles and Yunes-Jimenez, 2009; Iles, 2014).

Do acoustic differences between populations reflect ecological, genetic, and selection differences between populations?

My results indicate that cultural differences between populations influence acoustic variation. In particular, cultural drift affects acoustic variation both within and among populations for both male and female Rufous-and-white Wrens. In response to playback, Rufous-and-white Wrens respond more intensely to local songs (Hick *et al*., 2015), suggesting that local songs are selected for. My analysis of the songs of first-generation migrants corroborates this pattern: both male and female first-generation migrants learn local songs post-dispersal (Chapter 4). Songs may be influenced by cultural selection, where birds learn songs to match their neighbours (Payne, 1981) or they may be influenced by sexual selection, where birds learn local songs because mates preferentially choose individuals who sing them (Danner *et al.* 2013). Further studies are necessary to discriminate between these two processes.

The five sites where I recorded Rufous-and-white Wrens all differ ecologically based on vegetation and climate (Chapter 3). The Acoustic Adaptation Hypothesis states that acoustic signals

are optimized for transmission through the natural environment of the animals that produce them, and acoustic signals used for long-range transmission should exhibit adaptations that minimize degradation and maximize transmission (Morton, 1975). I found evidence to suggest that playback site influences the transmission of male and female syllables, but overall there is little evidence to suggest that either the syllables or whole songs of males and females are adapted for transmission through the different forest types I tested (Chapter 2). Additionally, I found no evidence to support the idea that ecological differences between populations drive patterns of geographic variation in male and female songs. One reason for this pattern may be that the songs of this species are already adapted for transmission through dense habitats such as forests (Barker *et al.*, 2009), and that microhabitat differences between the sites in our study were not as large as the differences for species living in more diverse habitat types (e.g. open fields vs. forests; Handford and Lougheed, 1991; Tubaro and Segura, 1994). While male and female songs showed similar transmission properties, my results suggest that there are some transmission differences between sexes; whereas male songs appear to be optimized for maximum transmission, female songs appear to be optimized for transmission through densely vegetated habitat. These results suggest that different pressures may influence the evolution of male and female songs (Price, 2015).

Genetic analyses reveal strong population structure in Rufous-and White Wrens (based on both microsatellite and mtDNA pairwise comparisons; Chapter 3), despite the close proximity of the five sites where I collected samples. Tropical species exhibit greater population structure than temperate species and this pattern is thought to arise due to reduced gene flow at tropical latitudes (Martin and McKay, 2004). Acoustic differences were not linked with microsatellite genetic differences or mtDNA genetic differences, as has been shown in other species (Wright and Wilkinson, 2001; Yoktan *et al.*, 2011; Ortiz-Ramírez *et al.*, 2016). While acoustic differences would not be linked directly with selectively neutral genetic differences, they may arise from limited gene

flow and increased isolation. Gene flow and isolation are known to enhance the effect of drift (Francisco *et al*., 2007; Smith *et al*., 2014), and therefore the observed genetic differences among my study sites may enhance the effects of cultural drift on acoustic variation in male and female Rufous-and-white Wrens.

Do patterns of acoustic variation relate to between-sex differences in dispersal and song learning strategies?

My analyses of immigration and dispersal provide insight into processes underlying vocal learning in male and female Rufous-and-white Wrens, such as the timing of vocal learning and the selection of potential tutors by young males and females. One of the goals of my dissertation was to examine the role of immigration on acoustic variation, and determine if young birds learn songs prior to dispersal from natal territories. My results indicate that young birds learn songs post-dispersal (Chapter 4); therefore, first-generation migrants do not appear to introduce songs from their natal population into their breeding populations. Furthermore, my analysis of spatial acoustic structure indicates a direct relationship between the degree of song sharing and distance between territories (Chapter 6), as has been shown in this species (Mennill and Vehrencamp, 2005) and other species (Tracey and Baker, 1999). As such, males learn fewer of their fathers' songs the farther they disperse from their natal territories. This result suggests that males match their vocal repertoires to those of their neighbours (Payne, 1981), possibly because song matching plays an important role in interactions with neighbours (Beecher *et al*., 2000). The degree of song sharing decreased with distance in females also, although this pattern was not as strong in females. Daughters showed no relationship between dispersal distance from natal territories and sharing with their mothers. These results may arise because females disperse farther than males and potentially learn from more tutors as they search for mates before settling on breeding territories.

Historically tropical birds have been thought to exhibit limited dispersal (Stutchbury and Morton, 2008), although recent studies have found that they are in fact capable of extensive dispersal (Van Houten, 2007). My estimates of natal dispersal show that Rufous-and-white Wrens are capable of dispersing substantial distances, and these estimates are likely conservative, given that I only recaptured 9% of the individuals banded as nestlings (Chapter 6). Furthermore analyses of population structure indicate that individuals do disperse between populations. Importantly, I demonstrate that dispersal is female-biased at both local scales and broad scales (Chapter 3 and 6). My results indicate that natal dispersal has a greater influence on both spatial acoustic structure and spatial genetic structure.

Future work

Recent work phylogentic and comparative analyses reveal that female song is more common than was previously believed (Odom *et al*., 2014; Price, 2015) and that 18% of all bird species use communal male-female acoustic signals such as duets or choruses (Tobias *et al*., 2016). For many species, the documented incidence of female song is purely descriptive and further studies are required to examine vocal output, acoustic structure, and acoustic variation in female songbirds (Riebel, 2003; Langmore *et al.,* 2005; Garmaszegi *et al*., 2007). The analyses conducted in my dissertation included populations from only a portion of the Rufous-and-white Wren range, and therefore additional studies should be conducted that include samples from a greater portion of this species' range, including across subspecies boundaries. More studies are also necessary in other species, because different evolutionary forces may influence acoustic divergence in other species.

Future studies should continue to examine individual variation in the songs of tropical birds. My study of song evolution showed that both song structure and song type frequencies change over time (Chapter 6). Future studies can build on these findings by examining whether song structure changes within individuals over time, and whether individuals use their repertoires consistently over

time. Additionally, studies of individual variation may benefit by incorporating physiological components (Gill *et al*., 2007; 2008), because physiological differences may help to explain differences in singing behaviour between individuals, including song output and song repertoire size. These studies could be especially informative on the singing behaviour of females, where there appears to be greater variation between individuals in song output and song repertoire size. Physiological data should also be compared at the population level, to determine whether there are physiological differences between populations, and the role any such differences play in the evolution of acoustic variation in birds.

Future studies should focus on the ontogeny of female song in addition to behavioural descriptions. While my work provides insight into the timing of vocal learning and the identity of potential song tutors, more work is necessary to better determine how songs develop in wild populations of male and especially female birds. In particular, how do animals like Rufous-and-white Wrens, which can learn up to 15 songs, decide on the songs they include in their repertoire, and how often and for how long do they listen to song tutors during the learning process?

Future studies should continue to examine genetic population structure in tropical birds, especially in species like Rufous-and-white Wrens that have wide distributions. Recent discoveries of two closely related sister species to Rufous-and-white Wrens (Niceforo's Wren, *Thryophilus nicefori*, and Antioquia Wren, *Thryophilus sernai*; Valderamma *et al*., 2007; Lara *et al*., 2012) indicate that a phylogeographic analysis of this species across the entirety of its distribution is necessary. A comparison of genetic variation in Rufous-and-white Wrens and these two sister species would help to expand our knowledge of the speciation process in birds. In particular, further comparisons of the songs of these three species offer a compelling system to examine the factors that promote acoustic divergence. Since females sing in all three species (Mennill and Vehrencamp, 2005; Valderamma *et al*., 2007; Lara *et al*., 2012), this system also offers opportunities to examine acoustic variation and

evolution in female songbirds. Lastly, future studies of tropical bird species should incorporate landscape genomics. Given that many tropical species inhabit ecologically diverse habitat these studies would provide greater insight into the environmental factors that influence local adaptations, and whether these adaptations have a genetic basis (Manel *et al*., 2013).

Conclusion

My work expands our knowledge of culture in both male and female songbirds, and indicates that while male and female cultural evolution share similarities, there are also important differences. My research indicates that cultural drift is a significant driver of acoustic evolution in both males and females. Additionally my research shows that females disperse farther than males from natal territories and these differences likely contribute to differences in spatial acoustic structure and spatial genetic structure between sexes. Overall my dissertation provides greater insight into the diversity and complexity of song in male and female tropical birds.

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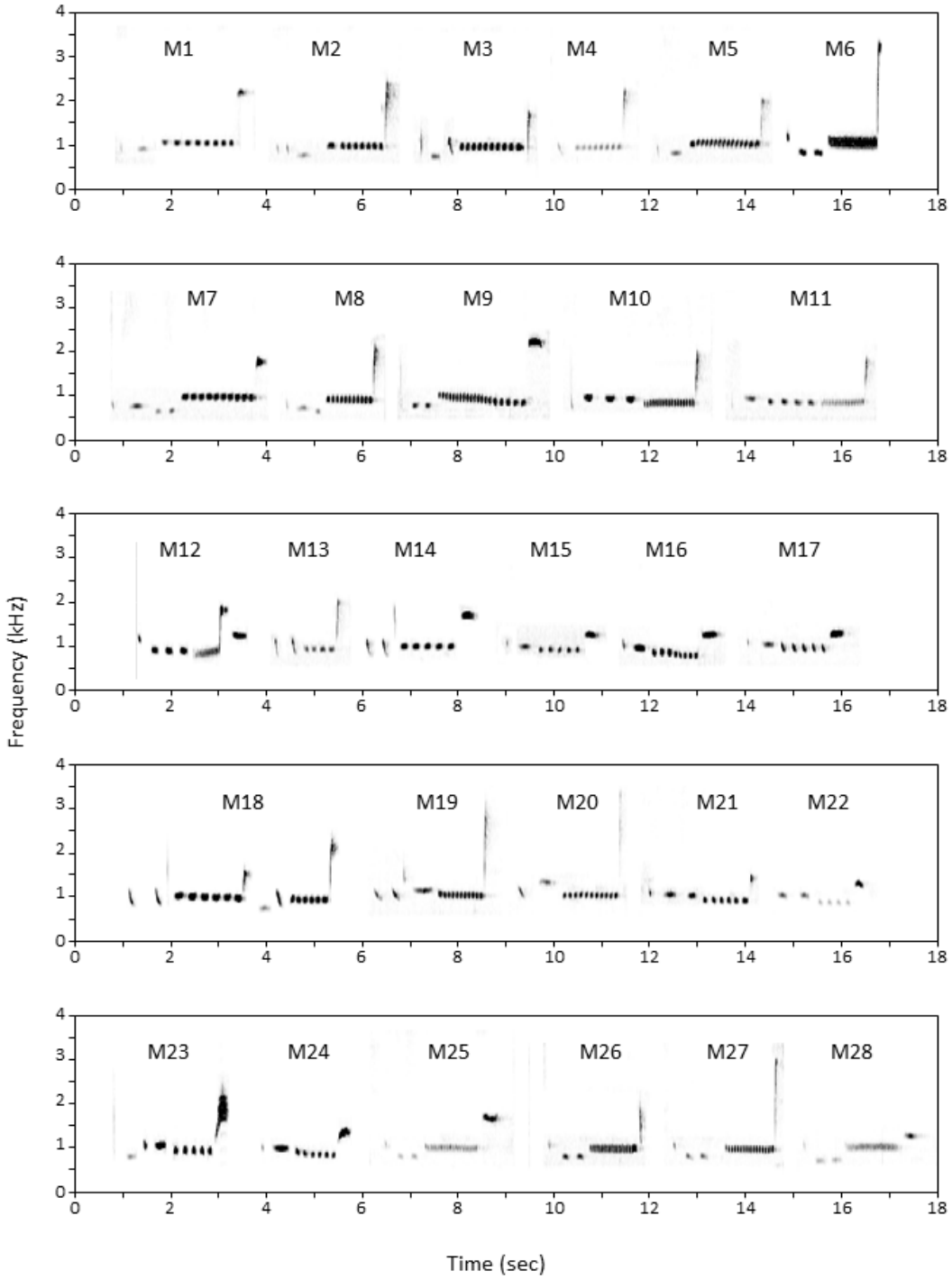
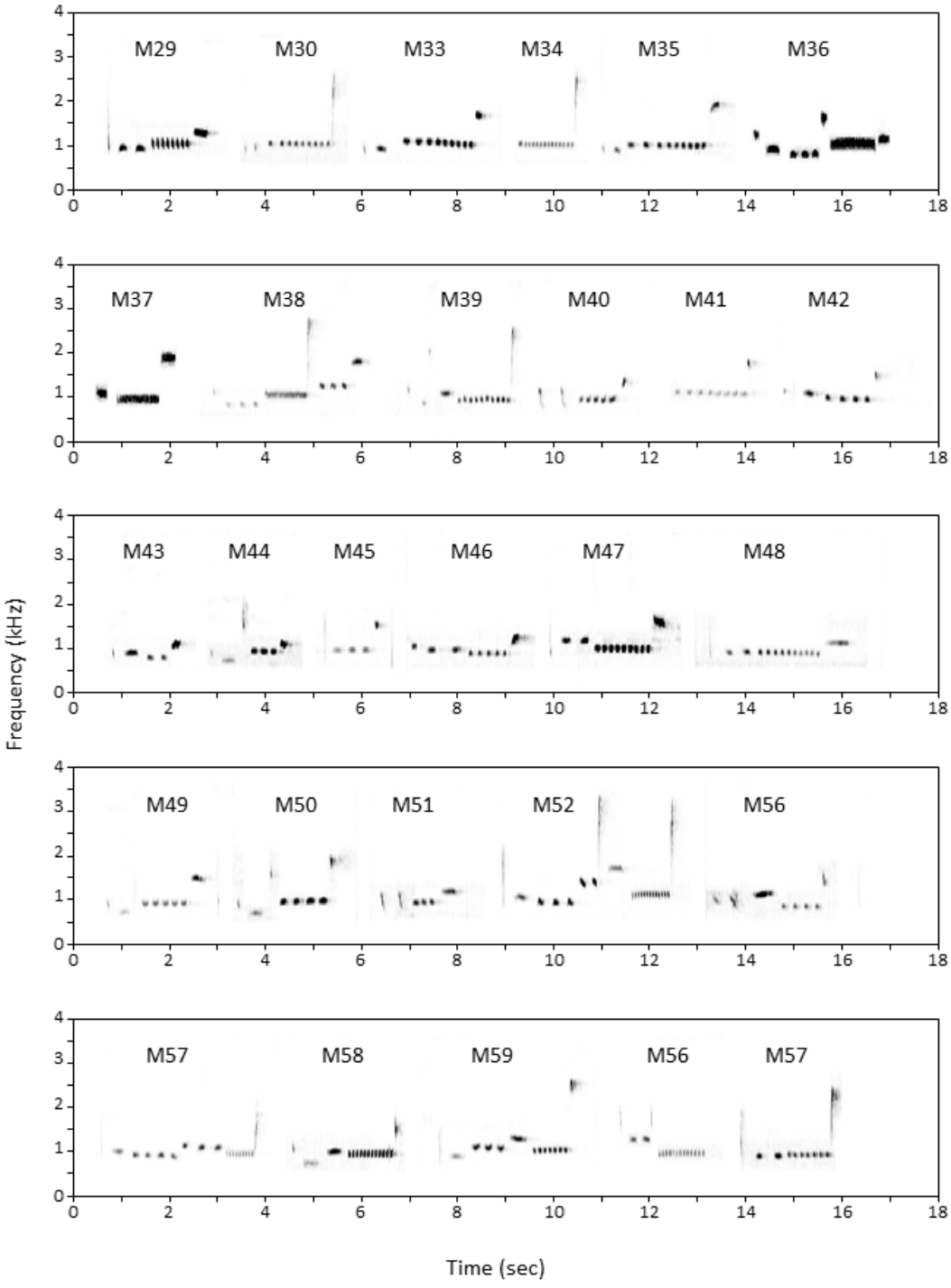
Appendix

Figure A1.1: Sound spectrograms of the 69 male Rufous-and-White Wren song types recorded from Santa Rosa from 2003 to 2013.

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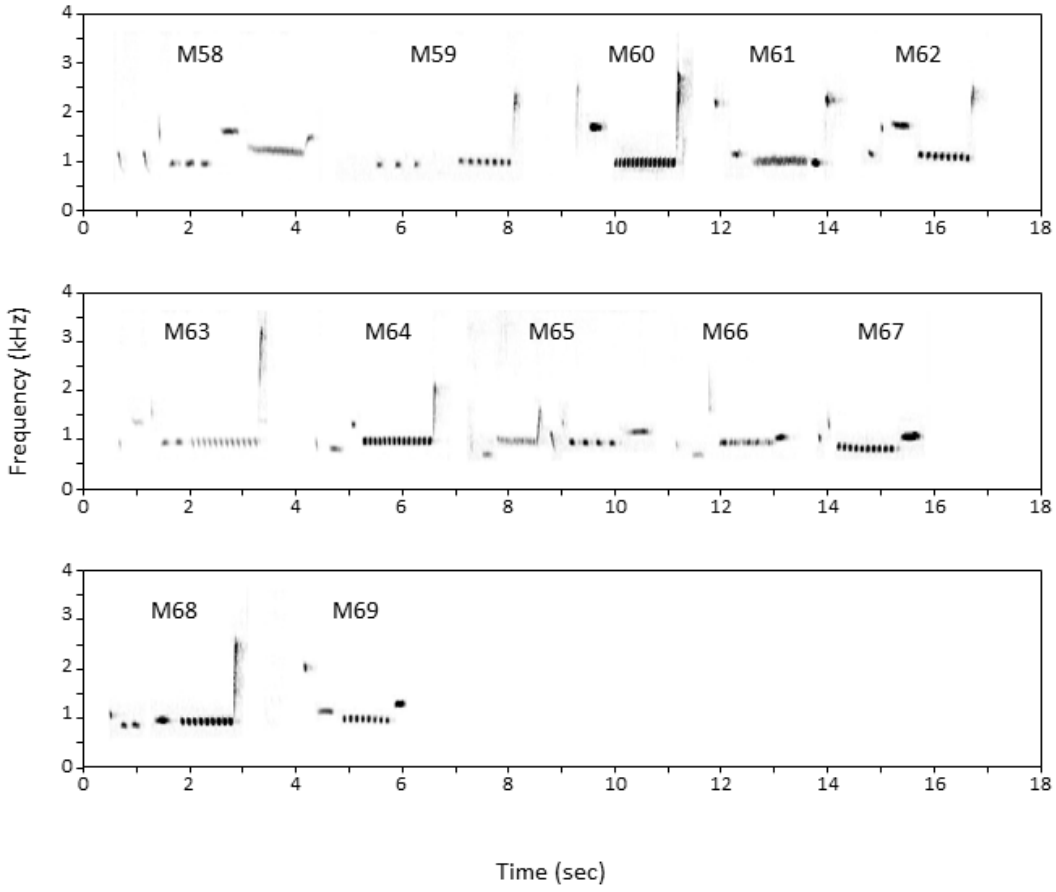
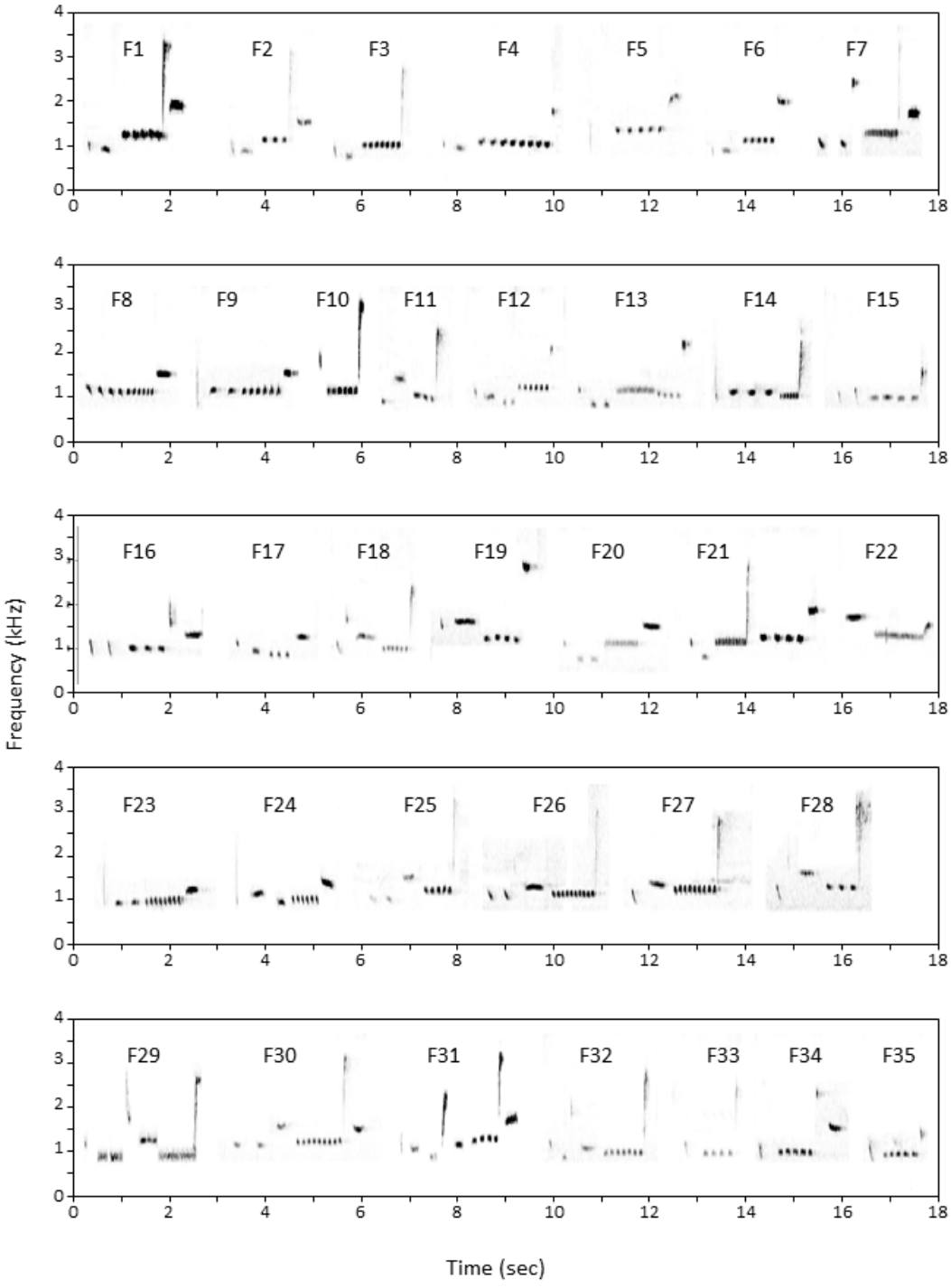


Figure A1.2: Sound spectrograms of the 59 female Rufous-and-White Wren song types recorded from Santa Rosa from 2003 to 2013.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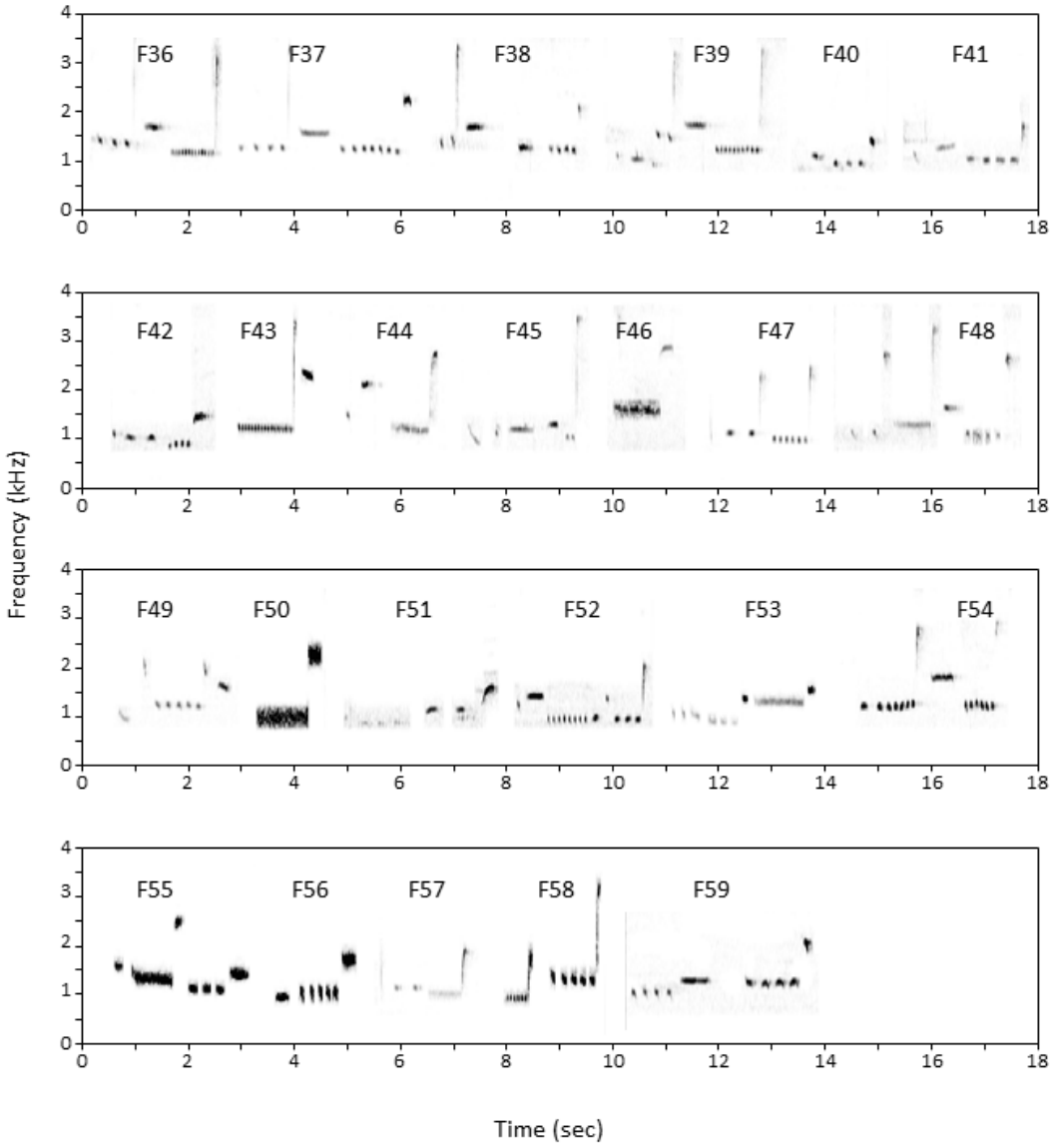
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